Applied Biosystems AutoCaller™ Software

SNP Genotyping Analysis Tool

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Contents

Preface

How to use this guide

Purpose of this guide

The *Applied Biosystems AutoCaller™ Software User Guide* provides step-by-step instructions for using the Applied Biosystems AutoCaller™ Software.

Audience

This guide is intended for novice and experienced AutoCaller software users at the following User Levels:

- Administrator
- Scientist
- Production

Assumptions

This guide uses conventions and terminology that assume a working knowledge of the Microsoft® Windows® operating system.

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: Users at the Administrator and Scientist User Levels are allowed to edit data. Users at the Production User Level are not allowed to edit data.

IMPORTANT! The AutoCaller software is intended for SNP genotyping experiments only. Be sure to open an Allelic Discrimination (AD) plate document.

Safety alert words

Safety alert words also appear in user documentation. For more information, see "Safety alert words" on page ix.

How to obtain more information

Documentation

AutoCaller software documentation

Portable document format (PDF) versions of this guide and the *Quick Reference Card: Applied Biosystems AutoCaller™ Software Tasks for Users at the Administrator User Level* (PN 4387768) are available on the AutoCaller software installation CD and from the Help menu in AutoCaller software.

Note: To open the user documentation included on the AutoCaller software installation CD, use the Adobe® Acrobat® Reader® software available from **www.adobe.com**.

Related documentation

Document	PN
Applied Biosystems 7900HT Fast Real-Time PCR System Allelic Discrimination Getting Started Guide	4364015
GeneAmp® PCR System 9700 Base Module: User's Manual	4303481
SDS Software Online Help	N/A
Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Genotyping Experiments	4376786
StepOne and StepOnePlus Software Online Help	N/A
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Genotyping Experiments	4387784
StepOne and StepOnePlus Software Online Help	N/A
OpenArray™ NT Imager Genotyping System User Guide	4400385
OpenArray NT Imager Genotyping Software Online Help	N/A

Note: For additional documentation, see "How to obtain support" on page vii.

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, go to **www.appliedbiosystems.com**, then click the link for **Support**. (See "How to obtain support" below).

How to obtain support

For the latest services and support information for all locations, go to **www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Safety Information

Safety conventions used in this document

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

Definitions

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

– Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

– Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

– Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Examples

The following examples show the use of safety alert words:

IMPORTANT! The sample name, run folder name, and path name, *combined*, can contain no more than 250 characters.

CAUTION **MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD.** These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

WARNING Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

CAUTION **MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD.** These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

1 Introduction

This chapter covers:

About the Applied Biosystems AutoCaller™ Software

Applied Biosystems AutoCaller™ Software is a SNP genotyping analysis tool and client-server program that you can use to efficiently analyze, edit, and compare TaqMan® SNP Genotyping Assays run on an the following Applied Biosystems Real-Time PCR Instruments:

- 7900HT Fast Real-Time PCR System
- 7500 Real-Time PCR System with 7500 software
- 7500 Fast Real-Time PCR System with 7500 software
- StepOne™ Real-Time PCR System
- StepOnePlus™ Real-Time PCR System
- OpenArray™ NT Imager Genotyping System

Features

AutoCaller software allows you to:

- Import SNP genotyping experiment data from SDS, EDS, and XML files, then manage the data in a database.
- Search the database for assays using specific search criteria.
- Easily view data in a variety of ways (plots, statistics, status codes, and so on).
- Edit data (your edits are saved to the database).
- Overlay data from multiple plates.
- Export data to:
  - Perform downstream/secondary analysis software tools
  - Share with other laboratories that use the AutoCaller software

Compatible data files

You can use AutoCaller software with the following data files:

- SDS files (for allelic discrimination plates only) created with the SDS Software for the Applied Biosystems 7900HT Fast Real-Time PCR System.

  When you set up your SDS files, you must include information that can be successfully accessed by the AutoCaller software (see "Set up an SDS file" on page 7).
- Text (*.txt) files created with a simple text application and formatted for use with the AutoCaller software.
- EDS files created on the Applied Biosystems StepOne, StepOnePlus, and 7500 and 7500 Fast Real-Time PCR Systems.
- Files exported from the OpenArray SNP Genotyping Analysis software.
- Files in *.xml format created with the AutoCaller software.

Notes

About user levels

Your AutoCaller software Administrator is responsible for managing users in the AutoCaller software. When creating a user, the Administrator must assign the user a User Level. By assigning a User Level, the Administrator can control the tasks each user is allowed to perform in the AutoCaller Software.

User level tasks

There are three predefined User Levels in the AutoCaller software. Users at each User Level can perform the tasks listed below:

User Level	Allowed Tasks
Administrator	• Install the AutoCaller software‡ • Manage users‡ • Manage studies (includes deleting studies)‡ • Manage assay information • Manage assay collections • View and edit data • Publish data • Manage sample sets • Manage *.xml files
Scientist	• Manage studies (does not include deleting studies) • Manage assay information • Manage assay collections • View and edit data • Publish data • Manage sample sets • Manage *.xml files
Production	• Manage assay collections • View data • Publish data

‡ For information on installing the AutoCaller software, managing users, and backing up and restoring the AutoCaller software database, see the *Quick Reference Card: Applied Biosystems AutoCaller™ Software Tasks for Users at the Administrator User Level*.

Notes

Workflow

This user guide explains how to perform tasks with the AutoCaller software, following the workflow below:

Preparation Tasks (Chapter 2)

1. Set up files for import into the AutoCaller software:
   - SDS Files
   - EDS Files
   - OpenArray Files
2. Log in to the AutoCaller software and (optionally) change your password.

▼

Manage Studies and Assay Information (Chapter 3)

1. Create a study and set the analysis criteria.
2. Import data files into a study.
3. Manage studies.
4. Edit assay information.
5. Edit assay collections.
6. Delete assays and plates.

▼

View and Edit Data (Chapter 4)

1. Search a study for assays.
2. View data.
3. Edit data.

▼

Publish Data (Chapter 5)

1. Review the report types.
2. Create reports from a study.
3. Create comparison reports.
4. Use reports in publications and/or downstream analysis.

Notes

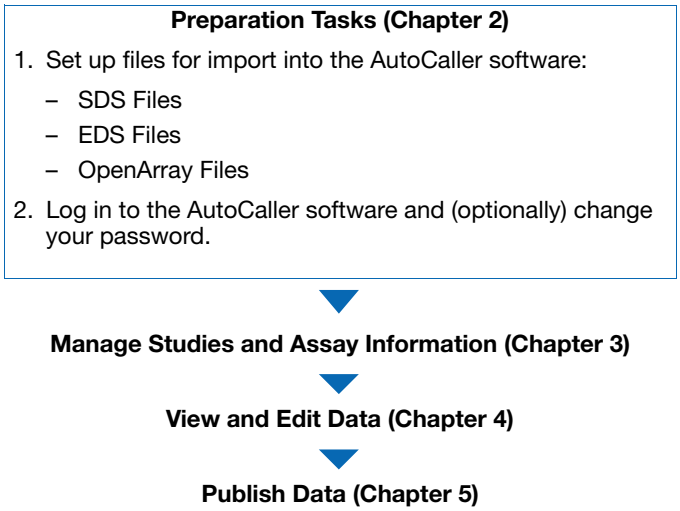
2 Preparation Tasks

This chapter covers:

Chapter overview

This chapter explains how to set up your SDS files for use with the Applied Biosystems AutoCaller™ Software and how to log in to the AutoCaller software and change your password.

Workflow



Required user levels

All User Levels in the AutoCaller software are allowed to perform the procedures in this chapter. For information on User Levels, see "About user levels" on page 3.

Notes

Set up an SDS file

About SDS files

An SDS file is an SDS software document that stores data for a single reaction plate. SDS files are also called *plate documents.* When you perform an experiment on an Applied Biosystems 7900HT Fast Real-Time PCR System, you:

1. Create an SDS file in the SDS software. The SDS file contains information about each well in the reaction plate, including the sample names and assay IDs.
2. Run the reaction plate on the 7900HT instrument. The data from the run is collected by the SDS software and stored in the SDS file.

After the run, you can import the SDS file into the AutoCaller software database. The AutoCaller software analyzes the SDS file data upon import; use the AutoCaller software to view and edit the data.

Using SDS files with the AutoCaller software

To ensure that the SDS file is imported correctly into the AutoCaller software database, set up the SDS file so that it includes the following information:

- Sample name
- Assay ID
- Correct information for the no template control (NTC) wells

  IMPORTANT! You must label all NTC wells **NTC**. If you label the NTC wells as water, blank, and so on, the AutoCaller software does not recognize them as NTC wells.

- Reaction plate name or barcode
- (*Optional*) AutoCaller software study information

Applied Biosystems recommends that you include the above information when you first create an SDS file. You can add this information to an existing SDS file. However, if you do not include this information before you import the SDS file into AutoCaller software, the AutoCaller software displays an error message and does not import the SDS file.

Set up an SDS file

You can set up:

- A new SDS file (page 7)
- An existing SDS file (page 11)

These procedures include only the steps required to set up an SDS file for import into the AutoCaller software. These procedures do *not* include all of the setup steps; for complete procedures, see the *Applied Biosystems 7900HT Fast Real-Time PCR System Allelic Discrimination Getting Started Guide.*

To set up a new SDS file:

1. Start the SDS software, then select **File** ▸ **New** to open the New Document dialog box.

Notes

2. From the Assay drop-down menu, select **Allelic Discrimination**.

   IMPORTANT! The AutoCaller software is intended for SNP genotyping experiments only. Be sure to create an Allelic Discrimination (AD) plate document.

3. From the Container drop-down menu, select the appropriate reaction plate.

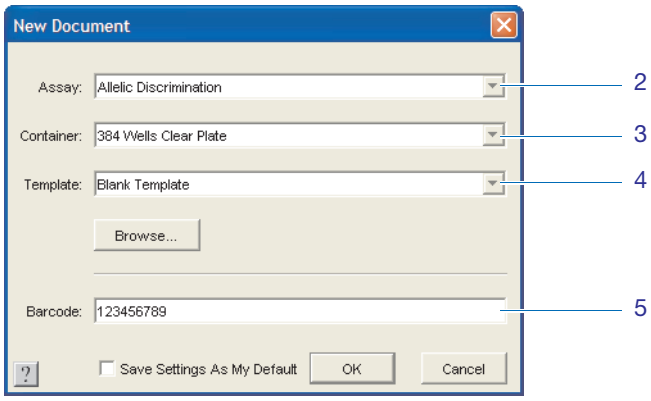
   Note: The TaqMan® DME Array is not a supported SNP genotyping application. Do not select the **384 Wells TaqMan Low Density Array** option from the drop-down menu.

4. Enter the sample names by selecting the appropriate template from the Template drop-down menu.

   Note: Alternatively, enter the sample names manually in the Setup tab of the SDS software main window or import the sample names via a tab-delimited text file (*.txt). For more information, see the *SDS Online Help*.

5. In the Barcode field, enter a name for the reaction plate or enter or scan the barcode.

6. Click **OK** to close the New Document window and open the SDS software main window.



7. Enter the assay ID:

   a. In the Setup tab, click **Add Marker**, then:

      - Select the assay name or ID number.

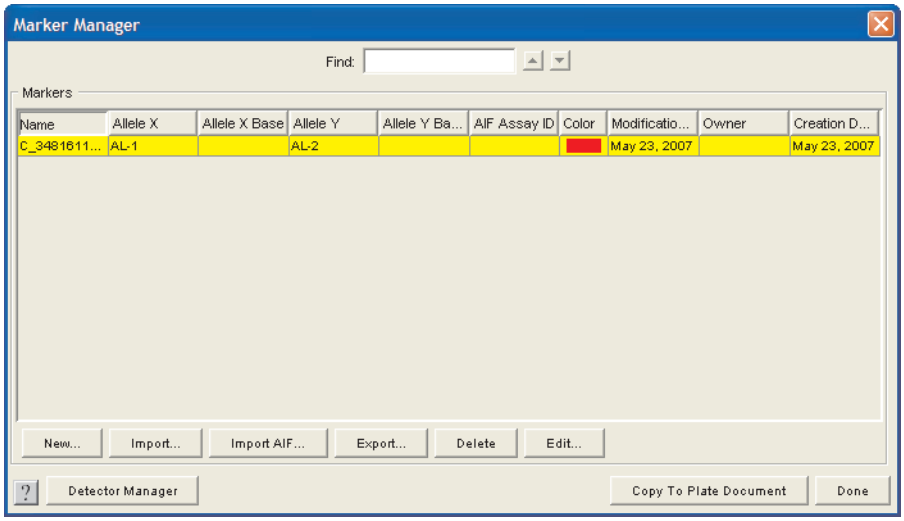
        or

      - Click **New**, enter the assay name or ID number, then assign the detectors.

        Note: In the SDS software, the term *marker* is used instead of *assay ID*. Both terms refer to the assay name or ID number. You can enter the Applied Biosystems assay identification number (for example, C_34816113_20), or a name of your own choosing.

Notes

b. Click **Done** to return to the SDS software main window.



8. Label the NTC wells correctly:

   a. In the plate layout, highlight the NTC wells.

   b. In the Setup tab, enter **NTC** in the Sample Name field.

IMPORTANT! You must label all NTC wells **NTC**. If you label the NTC wells as water, blank, and so on, the AutoCaller software does not recognize them as NTC wells.

Notes

9. (*Optional*) Enter study information. You can enter this information into each SDS file by typing or by copying and pasting.

   a. Select **Tools ▸ Document Info**.

   b. In the Plate Comments field, enter the following information:

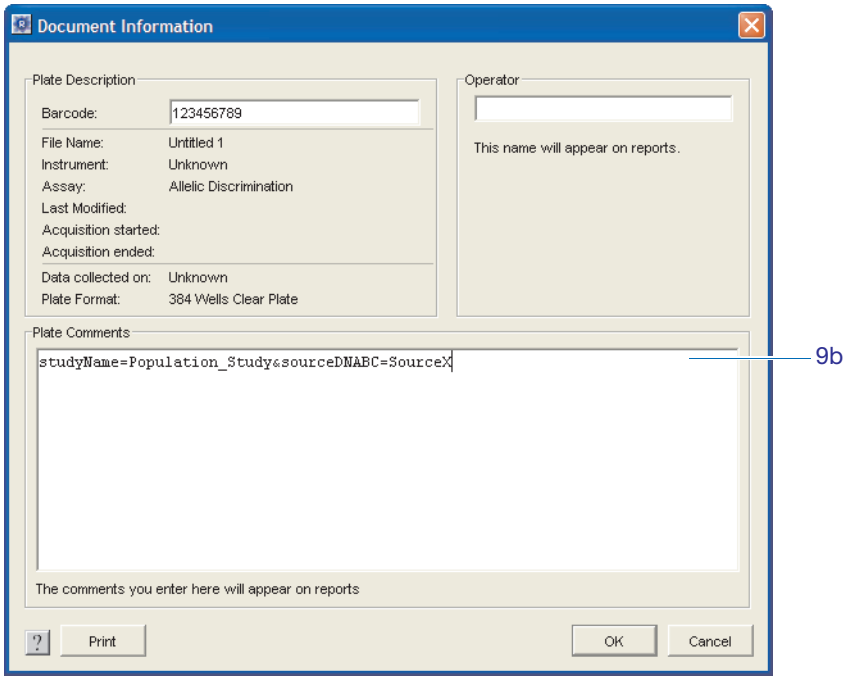
   studyName=<*study name*>&SourceDNABC=<*DNA plate name*>

   where:

   - *<study name>* is the AutoCaller study name that the SDS file will be imported into.
   - *<DNA plate name>* is the name of the source DNA plate (the DNA plate used to seed the reaction plate).

   For example, if your source DNA plate is named *SourceX* and you want to put this data into a study named *Population_Study*, you enter:

   studyName=Population_Study&sourceDNABC=SourceX.



   If you enter the *<study name>* in the SDS file, the AutoCaller software can automatically import the SDS file into the correct study. However, you can omit this step and manually import the SDS file into any study. When you manually import an SDS file, you can import the same SDS file into more than one study. See "Import SDS files" on page 24.

   c. Click **OK** to return to the SDS software main window.

10. Save and close the SDS file.

Notes

11. Run your reaction plate on the 7900HT instrument to collect data.

Note: Alternatively, you can perform thermal cycling on a GeneAmp® PCR System 9700, then use the 7900HT instrument to read the data. This option is best for users performing high-throughput experiments.

To set up an existing SDS file:

If you have already created and run an SDS file, follow this procedure to ensure the SDS file is compatible with the AutoCaller software.

1. Start the SDS software, then open an existing SDS file.

IMPORTANT! The AutoCaller software is intended for SNP genotyping experiments only. Be sure to open an Allelic Discrimination (AD) plate document.

2. Label the NTC wells correctly:
   a. In the plate layout, highlight the NTC wells.
   b. In the Setup tab, enter **NTC** in the Sample Name field.

IMPORTANT! You must label all NTC wells **NTC**. If you label the NTC wells as water, blank, and so on, the AutoCaller software does not recognize them as NTC wells.

3. Enter barcode and (*optional*) study information. You can enter this information into each SDS file by typing or by copying and pasting.
   a. Select **Tools ▸ Document Info**.
   b. In the Barcode field, enter a name for the reaction plate or enter or scan the barcode.
   c. (*Optional*) In the Plate Comments field, enter the following information:
      studyName=<*study name*>&SourceDNABC=<*DNA plate name*>
      where:
      - <*study name*> is the AutoCaller study name that the SDS file will be imported into.

Notes

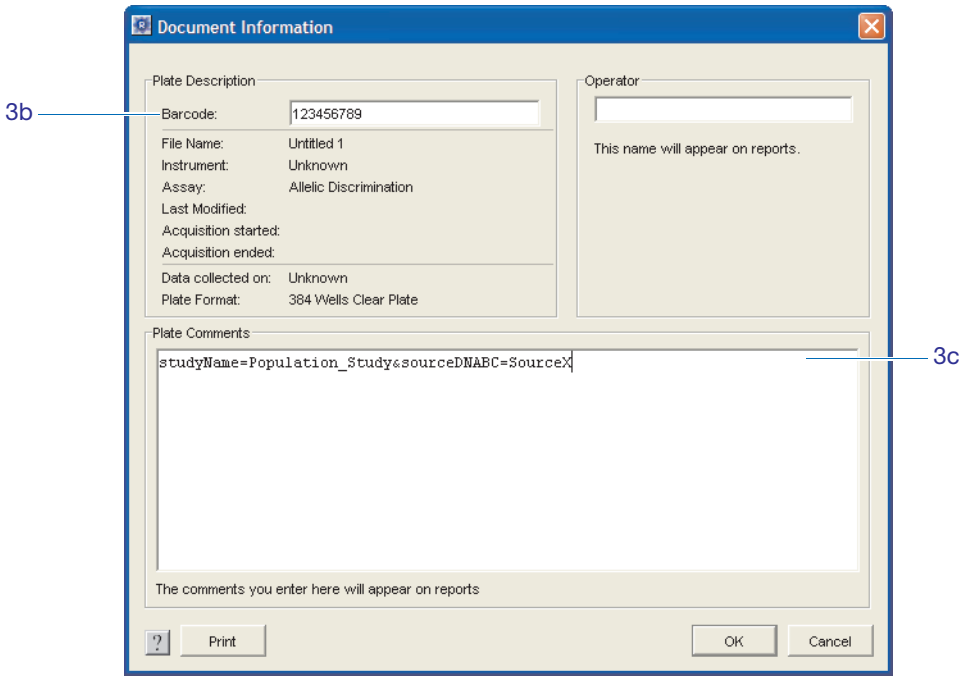
- *<DNA plate name>* is the name of the source DNA plate (that is, the name of the DNA plate used to seed the reaction plate).

For example, if your source DNA plate is named *SourceX* and you want to put this data into a study named *Population_Study*, you enter:

studyName=Population_Study&sourceDNABC=SourceX.

Note: If you enter the *<study name>* in the SDS file, the AutoCaller software can automatically import the SDS file into the correct study. However, you can omit this step and manually import the SDS file into any study. When you manually import an SDS file, you can import the same SDS file into more than one study. See "Import SDS files" on page 24.

d. Click **OK** to return to the SDS software main window.



4. Save and close the SDS file.

Notes

Set up an OpenArray™ file

About OpenArray™ data

AutoCaller software can read files created by the Applied Biosystems OpenArray™ SNP Genotyping Analysis software.

Export OpenArray™ data

To export OpenArray SNP Genotyping Analysis software data for use in AutoCaller Software:

1. Start the OpenArray SNP Genotyping Analysis software.
2. Open the project containing the data of interest.
3. Select **File ▸ Export To Applied Biosystems AutoCaller**.
4. Select a location for the file and click **Save**.

Set up an EDS file

About EDS files

An EDS file is a file created by the Applied Biosystems StepOne™, StepOnePlus™, 7500 or 7500 Fast Real-Time PCR system. An EDS file stores data for a single reaction plate. When you perform an experiment on any of these instruments, you:

1. Create an EDS file with information about each well in the reaction plate, including the sample names and assay IDs.
2. Run the reaction plate on the instrument. The data from the run is collected and stored in the EDS file.

After the run, you can import the EDS file into the AutoCaller software database. The AutoCaller software analyzes the EDS file data upon import; use the AutoCaller software to view and edit the data.

Note: Only 7500 and 7500 Fast instruments running 7500 Software v2.0 or greater create EDS files.

Using EDS files with the AutoCaller software

To ensure that the EDS file is imported correctly into the AutoCaller software database, set up the file so that it includes the following information:

- Sample name
- Assay ID – Either imported from the Assay Information file (AIF) or entered manually
- Correct information for the negative control wells

  IMPORTANT! You must label all negative control wells. If you label the negative control wells as water, blank, and so on, the AutoCaller software does not recognize them as negative control wells.
- Reaction plate name or barcode

Notes

- (*Optional*) AutoCaller software study information

Applied Biosystems recommends that you include the above information when you first create an EDS file. If needed, however, you can add this information to an existing EDS file or you can enter it when you load the file into AutoCaller software.

Set up an EDS file

Note: The following information only includes instructions for the AutoCaller software-specific information you must include when designing an experiment in StepOne and 7500 Software v2.0. See the appropriate Getting Started Guide for detailed information on designing genotyping experiments.

In the Experiment Properties screen, be sure to:

- Click the **Barcode** field and enter a name for the reaction plate or enter or scan the barcode.
- Click **Genotyping** for Experiment Type.
- (*Optional*) In the Comments field, enter the following information:

  studyName=<*study name*>&SourceDNABC=<*DNA plate name*>

  where:

  <*study name*> is the AutoCaller study name that the SDS file will be imported into.

  <*DNA plate name*> is the name of the source DNA plate (that is, the name of the DNA plate used to seed the reaction plate).

  For example, if your source DNA plate is named *SourceX* and you want to put this data into a study named *Population_Study*, you enter:

  studyName=Population_Study&sourceDNABC=SourceX.

  If you enter the <*study name*> in the EDS file, the AutoCaller software can automatically import the EDS file into the correct study. However, you can omit this step and manually import the EDS file into any study. When you manually import an EDS file, you can import the same EDS file into more than one study.

In the Samples and Replicates screen, be sure to include negative controls.

Follow the instructions in "Import EDS files" on page 29 to import the file into AutoCaller software.

Notes

Log In to the AutoCaller™ software

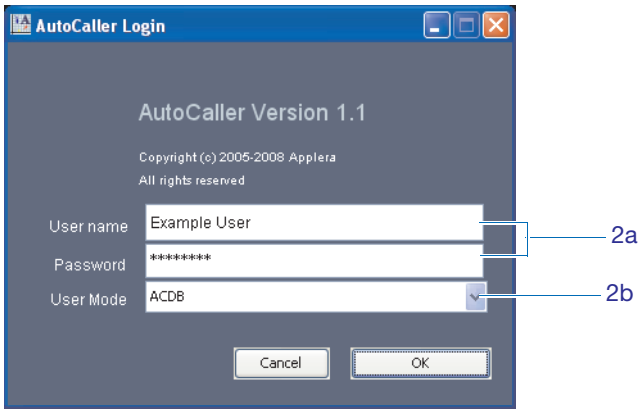
Log in

1. Double-click (AutoCaller software shortcut icon) to start the AutoCaller software. The software displays a DOS command window, the splash screen, then the AutoCaller Login dialog box.

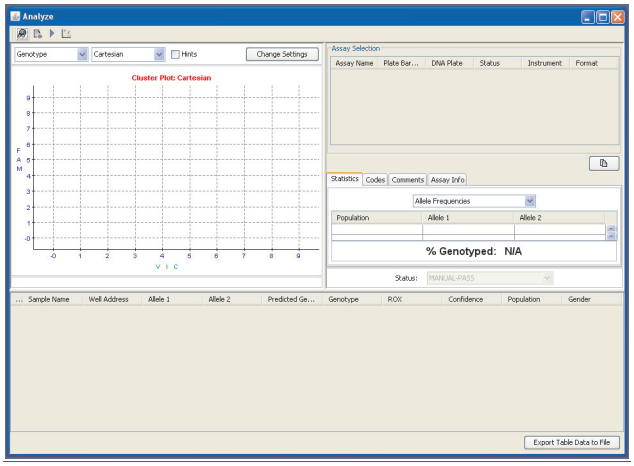
2. Complete the AutoCaller Login dialog box:

   a. In the User name and Password fields, enter the user name and password provided by your AutoCaller software Administrator.

   b. From the User Mode drop-down menu, select the name of the database. Be default, this is *ACDB*.



3. Click **OK** to open the AutoCaller software. By default, the AutoCaller software displays the Analyze window.



IMPORTANT! The remaining procedures in this guide assume that you have logged into the AutoCaller software.

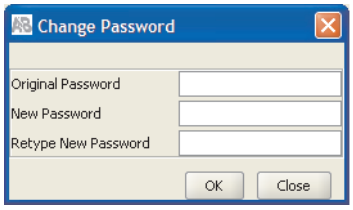
Notes

(Optional) Change your password

You can change your password at any time.

1. From the main window toolbar, select **Tools ▸ Change Password**.

2. Complete the Change Password dialog box:
   a. Enter your original password.
   b. Enter a new password.
   c. Enter the new password again to confirm it.



3. Click **OK**.
4. At the prompt, click **OK** to change your password.

Notes

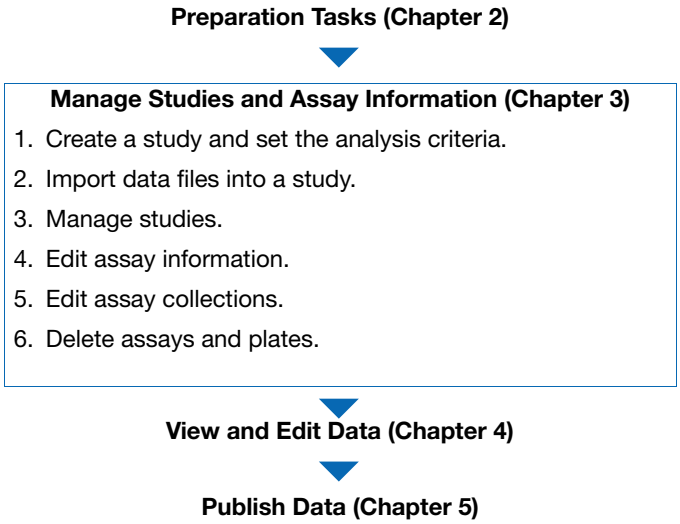
3 Manage Studies and Assay Information

This chapter covers:

Chapter overview

This chapter explains how to create a study in the Applied Biosystems AutoCaller™ Software, then import data files into the study. This chapter also explains how to manage studies and assays once they have been imported into the AutoCaller software database.

Workflow



Required user level

To perform the procedures in this chapter, log in to the AutoCaller software at the Administrator or Scientist User Level. For information on User Levels, see "About user levels" on page 3.

Notes

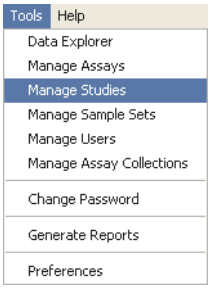
Create studies

In AutoCaller software, a *study* consists of a set of DNA samples run against a number of different TaqMan® genotyping assays.

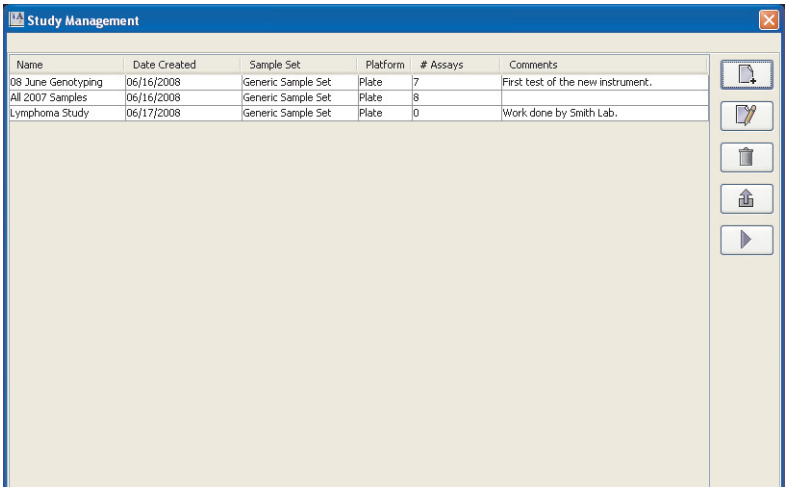
You can create multiple studies in the AutoCaller software database, organizing data based on projects or experiments. For example, you may want to create a validation study that includes all of your validation runs, and create separate studies for other experiments.

Create a study

1. From the main window toolbar, select **Tools** ▸ **Manage Studies**.



2. In the Study Management window, click  **Add a new study to the database** to access the Study Creation dialog box.



3. In the Study Creation dialog box, enter basic study information:

   a. In the Enter Study Name field, enter a name for your study that is descriptive and easy to remember.

   b. From the Choose Platform drop-down menu, select **Plate** to indicate that you are using a 96- or 384-well reaction plate or **OpenArray** to indicate you are using data collected from the OpenArray™ NT Imager Genotyping System.

   Note: You cannot mix data with different platforms in a single study.

Notes

c. From the Choose Sample Set drop-down menu, select:
   - **Generic Sample Set** if you have not imported any sample set files into the AutoCaller software or you do not want to use sample set files for this study.
   - The appropriate sample set if you have already imported sample set files into your study.

Note: To use sample sets, you must first create sample set files, then import the files into the AutoCaller software database. Sample set files contain information about each sample in a reaction plate. For more information on sample set files, see Appendix A on page 103.

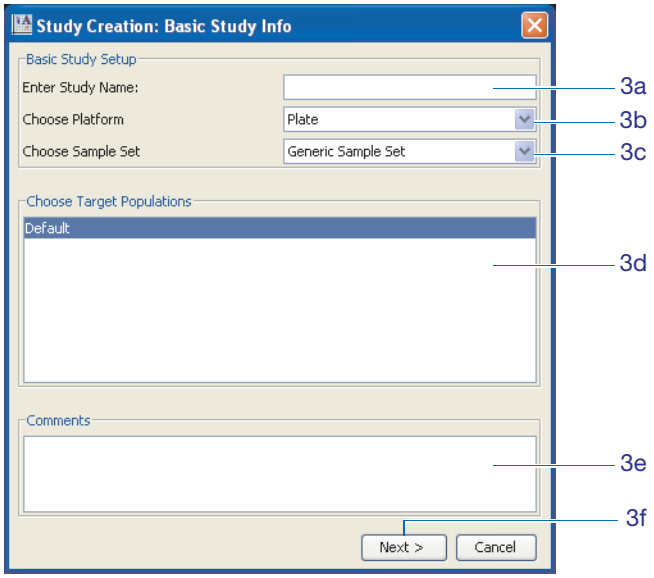
d. In the Choose Target Populations pane:
   - If you selected Generic Sample Set in step c, select **Default**. (This is the only option available for the Generic Sample Set.)
   - If you selected a sample set in step c, the populations identified for that sample set are displayed. Select one or more populations.

Note: Press **Ctrl+click** or **Shift+click** to select multiple populations.

The software calculates statistics for the selected populations. The calculated statistics appear in the Statistics tab of the Analysis window. For more information, see "Statistics tab" on page 67.

e. *(Optional)* In the Comments field, enter comments for this study.

f. Click **Next**.



Notes

4. Complete the Automation Settings dialog box:

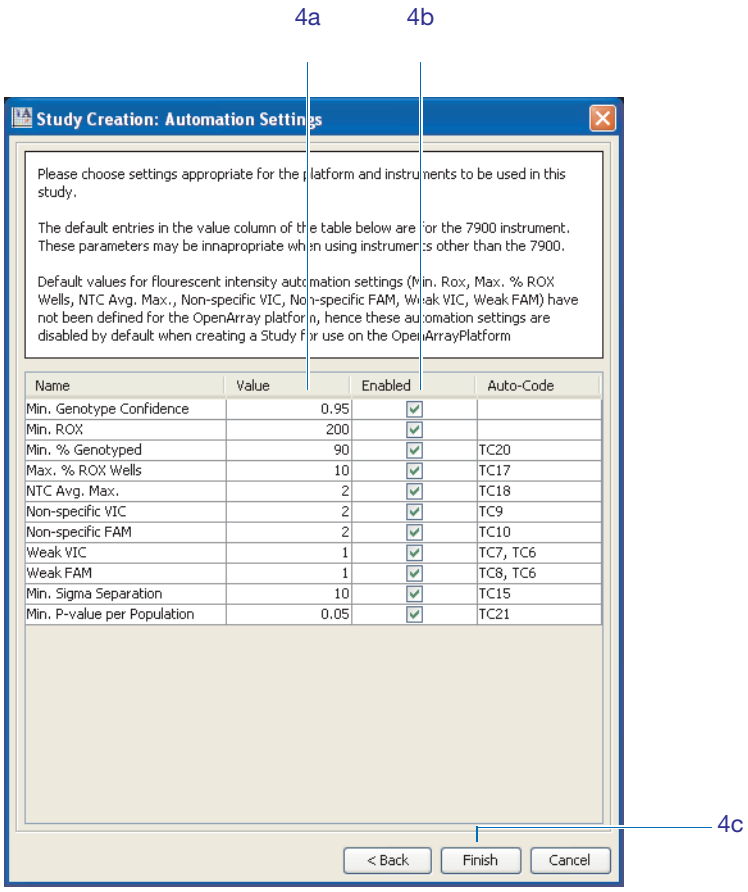
   a. To edit a value for a criterion, double-click the **Value** cell, then enter the desired value.

   Note: Applied Biosystems recommends accepting the default values for the analysis criteria.

   b. If you want the software to:
   - Apply a criterion to the study, select the **Enabled** check box.
   - Ignore a criterion, deselect the **Enabled** check box.

Note: The types of analysis criteria (Name column) and the Auto-Codes are predefined in the AutoCaller software and cannot be edited. For more information, see "About the automation settings" on page 22.

   c. Click **Finish**.



5. At the prompts, click **OK** to save the new study.

6. Click **Close** to close the Study Management window.

Notes

About the automation settings

Use the Automation Settings dialog box (displayed when you create or edit a study) to set the analysis criteria that you want applied to the current study when you import your SDS files into the study.

IMPORTANT! Analysis criteria are study-specific. The criteria are applied only to the current study and are not applied to any other studies in the AutoCaller software database. If you do not change the analysis criteria for a study, the software uses the default criteria (listed on page 22).

The Automation Settings dialog box displays the following:

Column	Description
Name	The name of each analysis criterion that you can apply to your study. For a description of each criterion, see page 22.
Value	The cut-off value defined for each analysis criterion. You can edit the value for each criterion. **Note:** Applied Biosystems recommends accepting the default values for the analysis criteria.
Enabled	Check boxes that you select or deselect to determine whether or not the AutoCaller software applies the analysis criteria to your study.
Auto-Code	The code automatically or manually assigned to an assay. Each code corresponds to a particular analysis criterion. For example, if your search returns an assay that lists the Auto-Code TC20, then that assay did not pass the Min % Genotyped analysis criterion. Auto-Codes and the corresponding analysis criteria are shown in the table below. **Note:** You can search for assays based on an Auto-Code(s). For more information, see "Search a study for assays" on page 57. **Note:** The AutoCaller software automatically assigns Auto-Codes to assays when a file is imported. Users at the Administrator or Scientist User Level can also assign (add or remove) Auto-Codes manually; for more information, see "Edit data" on page 73.

Predefined analysis criteria and auto-codes

Criterion	Auto-Code	Description
Min. Genotype Confidence	N/A	Minimum confidence value. The confidence value indicates the likelihood that the point belongs to the cluster (the cluster is defined by the software). Samples with values less than the specified minimum are genotyped as *UDT* (undetermined or 00).
Min. ROX	N/A	Minimum ROX™ dye value. Samples with values less than the specified minimum are flagged as *Low ROX*. ROX dye is a component of the TaqMan® master mix used in TaqMan® genotyping assays. A low ROX dye value may indicate mixing or pipetting errors. Samples flagged as *Low ROX* are not genotyped and cannot be edited.

Notes

Criterion	Auto-Code	Description
Min. % Genotyped	TC20	Minimum percentage of samples that must be genotyped for each assay. Assays with values less than the specified minimum are flagged.
Max. % ROX Wells	TC17	Maximum percentage of samples that can have a low ROX™ dye value (see "Min. ROX" above). Assays with values greater than the specified maximum are flagged. A high percentage of samples with low ROX dye values may indicate mixing or pipetting errors.
NTC Avg. Max	TC18	Maximum amount (as an average) of signal (FAM™ or VIC® dye) allowed for no template controls (NTCs). Assays with high NTC signal values are flagged. High NTC signal values may indicate contamination of the NTC well(s).
Non-specific VIC	TC9	Maximum amount of non-specific VIC dye signal allowed in the homozygous FAM dye cluster. Assays where the average signal value is greater than the specified maximum are flagged. In a perfect assay, samples (points) in the FAM dye cluster have an average VIC dye Rn value of 0. Values greater than the specified maximum may indicate non-specific probe-binding and cleavage.
Non-specific FAM	TC10	Maximum amount of non-specific FAM dye signal allowed in the homozygous VIC dye cluster. Assays where the average signal value is greater than the specified maximum are flagged. In a perfect assay, samples (points) in the VIC dye cluster have an average FAM dye Rn value of 0. Values greater than the specified maximum may indicate non-specific probe binding and cleavage.
Weak VIC	TC7, TC6	Minimum (as an average) Rn value of the VIC dye homozygote. Assays with average values less than the specified minimum are flagged with TC7. If both VIC and FAM Rn values are less than the specified minimum, assays are flagged with TC6.
Weak FAM	TC8, TC6	Minimum (as an average) Rn value of the FAM dye homozygote. Assays with average values less than the specified minimum are flagged with TC8. If both VIC and FAM Rn values are less than the specified minimum, assays are flagged with TC6.
Min. Sigma Separation	TC15	Minimum measure of cluster separation and cluster tightness. Assays with values less than the specified minimum are flagged.
Min. P-value Per Population	TC21	Minimum p-value for each population. Assays for which the samples have a p-value < 0.05 are 95% likely to have genotype frequencies that do not conform to Hardy-Weinberg Equilibrium (HWE) expectations. That is, there are too few homozygotes or too few heterozygotes.

Notes

Import files

Import SDS files

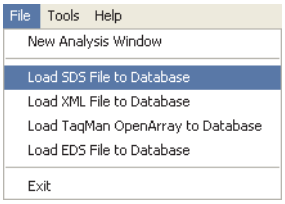
When you import SDS files created for allelic discrimination plates in the 7900HT System SDS software into the AutoCaller software database, the software:

- Imports all run data associated with the assays directly into a study. You can use the software to search studies for assays, then view and edit the assay run data. For more information, see Chapter 4 on page 55.
- Imports the assay names/ID numbers into the Assay Management window. For more information, see "Edit assay information" on page 41.

IMPORTANT! Before you import your SDS files, set up the SDS files to include information that can be successfully accessed by the AutoCaller software (see "Set up an SDS file" on page 7).

Import an SDS file

1. From the main window toolbar, select **File ▸ Load SDS File to Database** to open the Load Setup dialog box for importing SDS files.



2. Choose an Auto-analysis option. When an SDS file is successfully imported, the AutoCaller software automatically calls the genotypes for each assay in the file. In the Load Setup dialog box:
   - Deselect the **Conduct Auto-analysis** check box to track which assays are new. (All assays from the SDS file are given the status *NEW*; for more information, see "Assay Selection pane" on page 60.)
   - Select the **Conduct Auto-analysis** check box to automatically apply Auto-Codes (page 22) to each assay in the SDS file.
3. Select the study to import. From the Choose Study drop-down menu in the Load Setup dialog box, select one of the following:

Notes

- Select **Use study indicated in the file** to import the SDS file into the *<study name>* indicated in the SDS file (see "Set up an SDS file" on page 7). This option allows you to import multiple SDS files into multiple studies at the same time. For example, if you select a folder in step 4 below, the AutoCaller software automatically imports each SDS file into the *<study name>* indicated in that SDS file, even if all of the SDS files indicate different studies.

  Note: If you select **Use study indicated in the file** and the SDS file does not include a study name, the AutoCaller software displays an error message and does not import the SDS file.

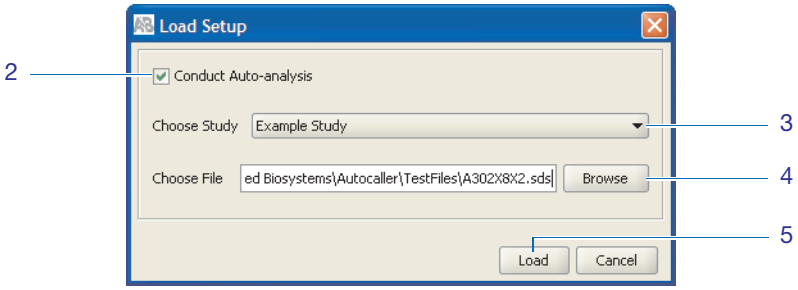
- Select the study you want the SDS file imported into.

  Note: You can import the same SDS file into more than one study.

4. Click **Browse** to navigate to and select the file or folder you want to import. The file or folder name is displayed in the Choose File field.

   Note: If you select a folder, all SDS files in the folder are imported.

5. Click **Load** to import the selected file or folder into the AutoCaller software. The Loading dialog box appears while the file is being imported.



Notes

6. When the File Progress field in the Loading dialog box displays *Done*:

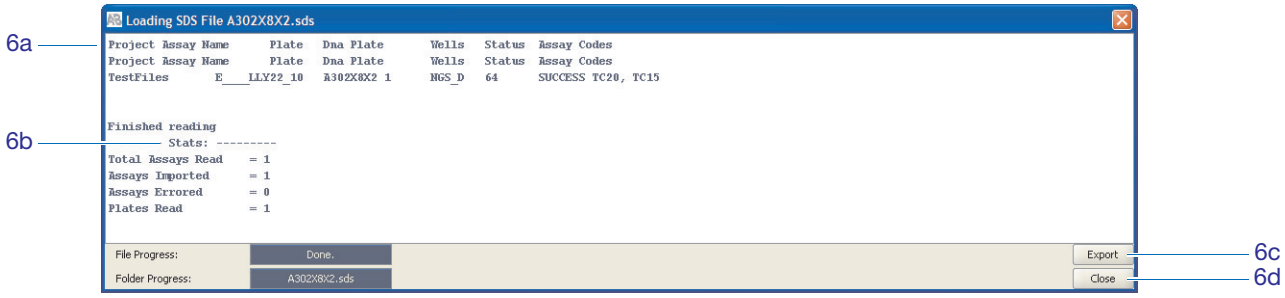
   a. Review the information for each assay:

Column	Description
Assay Name	The assay name or ID number.
Plate	The name or barcode of the reaction plate in which the assay was run. The name or barcode is entered by the user in the SDS file (page 7).
DNA Plate	The name or barcode of the source DNA plate that was used to seed the reaction plate: • *<DNA plate name>* – The software displays the source DNA plate name if the user included the name in the file (page 7) or in the sample set file (page 105). • *No DNA Plate* – The software displays *No DNA Plate* for any reaction plates that do not have a name or multiplate assigned.
Wells	The number of wells in the reaction plate containing sample/assay.
Status	SUCCESS or ERROR
Assay Codes	The Auto-Code(s) automatically or manually assigned to an assay, as defined by the analysis criteria. For a list of Auto-Codes and the corresponding analysis criteria, see page 22.

   b. Review the statistics information (Stats):
      - If the Assays Errored field displays 0, the SDS file(s) were imported successfully.
      - If the Assays Errored field displays >0, some or all of the SDS file(s) were not imported. See "Importing errors" on page 32.

   c. (*Optional*) Click **Export** to export and archive the information in the Loading dialog box.

   d. Click **Close**.



On import, the AutoCaller software automatically analyzes the data in the SDS files. To search for assays and view/edit the data, see Chapter 4 on page 55.

Notes

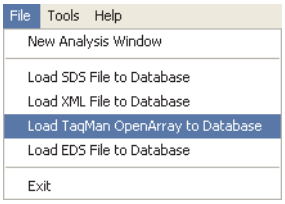
Import OpenArray™ NT Imager Genotyping System files

When you import OpenArray files created for allelic discrimination plates in the OpenArray™ NT Imager Genotyping System into the AutoCaller software database, the software:

- Imports all run data associated with the assays directly into a study. You can use the software to search studies for assays, then view and edit the assay run data. For more information, see Chapter 4 on page 55.
- Imports the assay names or ID numbers into the Assay Management window. For more information, see "Edit assay information" on page 41.

Import an OpenArray™ file

1. From the main window toolbar, select **File ▸ Load TaqMan OpenArray File to Database** to open the Load Setup dialog box for importing OpenArray files.



2. Choose an Auto-Analysis option. When a file is successfully imported, the AutoCaller software automatically calls the genotypes for each assay in the file. In the Load Setup dialog box:
   - Deselect the **Conduct Auto-analysis** check box to track which assays are new. (All assays from the file are given the status *NEW*; for more information, see "Assay Selection pane" on page 60.)
   - Select the **Conduct Auto-analysis** check box to automatically apply Auto-Codes (page 22) to each assay in the file.
3. From the Choose Study drop-down menu, select the study you want the Open Array file imported into.

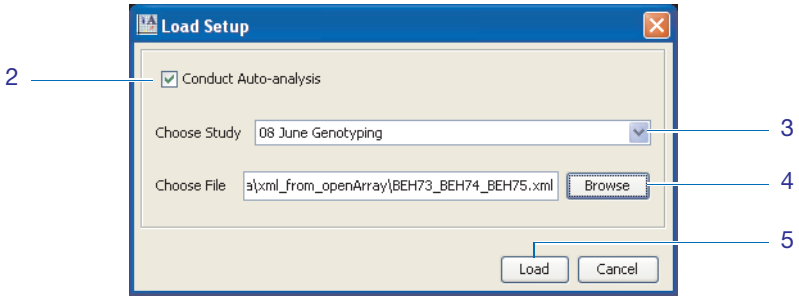
   Note: You can import the same OpenArray file into more than one study.

4. Click **Browse** to navigate to and select the file or folder you want to import. The file or folder name is displayed in the Choose File field.

   Note: If you select a folder, all files in the folder are imported.

Notes

5. Click **Load** to import the selected file or folder into the AutoCaller software. The Loading dialog box appears while the file is being imported.



6. When the File Progress field in the Loading dialog box displays *Done*:

   a. Review the information for each assay:

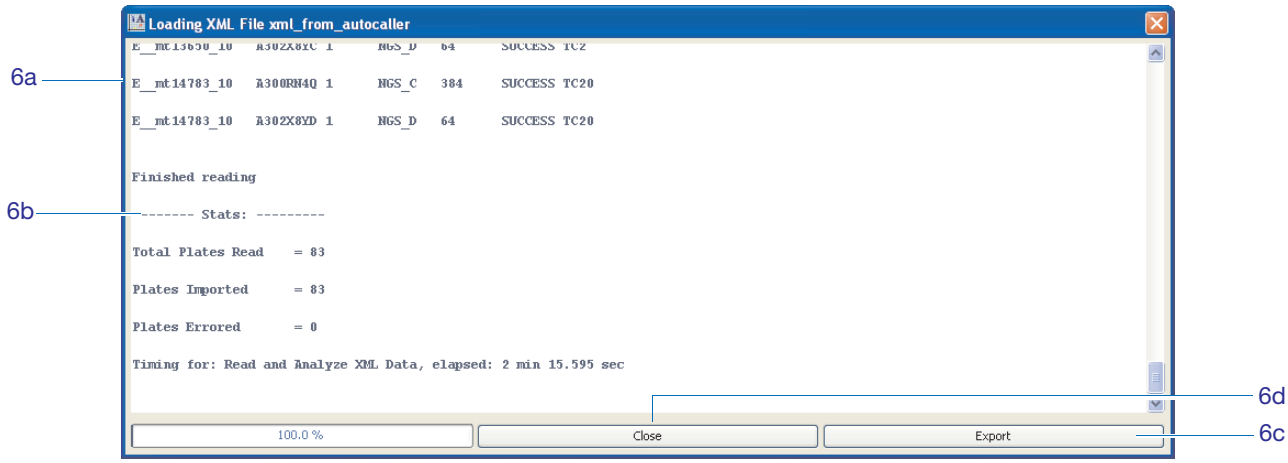
Column	Description
Assay Name	The assay name or ID number.
Plate	The name or barcode of the reaction plate in which the assay was run. The name or barcode is entered by the user in the file (page 7).
DNA Plate	The name or barcode of the source DNA plate that was used to seed the reaction plate: • *<DNA plate name>* – The software displays the source DNA plate name if the user included the name in the file (page 7) or in the sample set file (page 105). • *No DNA Plate* – The software displays *No DNA Plate* for any reaction plates that do not have a name or multiplate assigned.
Wells	The number of wells in the reaction plate containing sample/assay.
Status	SUCCESS or ERROR
Assay Codes	The Auto-Code(s) automatically or manually assigned to an assay, as defined by the analysis criteria. For a list of Auto-Codes and the corresponding analysis criteria, see page 22.

   b. Review the statistics information (Stats):
      - If the Assays Errored field displays 0, the OpenArray file(s) were imported successfully.
      - If the Assays Errored field displays >0, some or all of the OpenArray file(s) were not imported. See "Importing errors" on page 32.

   c. (*Optional*) Click **Export** to export and archive the information in the Loading dialog box.

   d. Click **Close**.

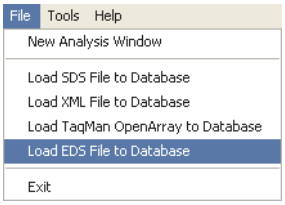
Notes



On import, the AutoCaller software automatically analyzes the data in the OpenArray files. To search for assays and view/edit the data, see Chapter 4 on page 55.

Import EDS files

1. From the main window toolbar, select **File ▸ Load EDS File to Database** to open the Load Setup dialog box for importing EDS files.



2. Choose an Auto-Analysis option. When a file is successfully imported, the AutoCaller software automatically calls the genotypes for each assay in the file. In the Load Setup dialog box:
   - Deselect the **Conduct Auto-analysis** check box to track which assays are new. (All assays from the file are given the status *NEW*; for more information, see "Assay Selection pane" on page 60.)
   - Select the **Conduct Auto-analysis** check box to automatically apply Auto-Codes (page 22) to each assay in the EDS file.

3. From the Choose Study drop-down menu, select one of the following:

Notes

- Select **Use study indicated in the file** to import the EDS file into the <*study name*> indicated in the EDS file (see "Set up an SDS file" on page 7). This option allows you to import multiple EDS files into multiple studies at the same time. For example, if you select a folder in step 4 below, the AutoCaller software automatically imports each EDS file into the <*study name*> indicated in that EDS file, even if all of the EDS files indicate different studies.

  Note: If you select **Use study indicated in the file** and the EDS file does not include a study name, the AutoCaller software displays an error message and does not import the EDS file.

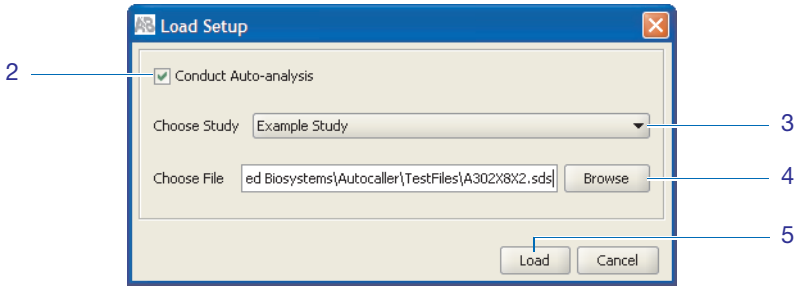
- Select the study you want the EDS file imported into.

  Note: You can import the same EDS file into more than one study.

4. Click **Browse** to navigate to and select the file or folder to import. The file or folder name is displayed in the Choose File field.

   Note: If you select a folder, all files in the folder are imported.

5. Click **Load** to import the selected file or folder into the AutoCaller software. The Loading dialog box appears while the file is being imported.



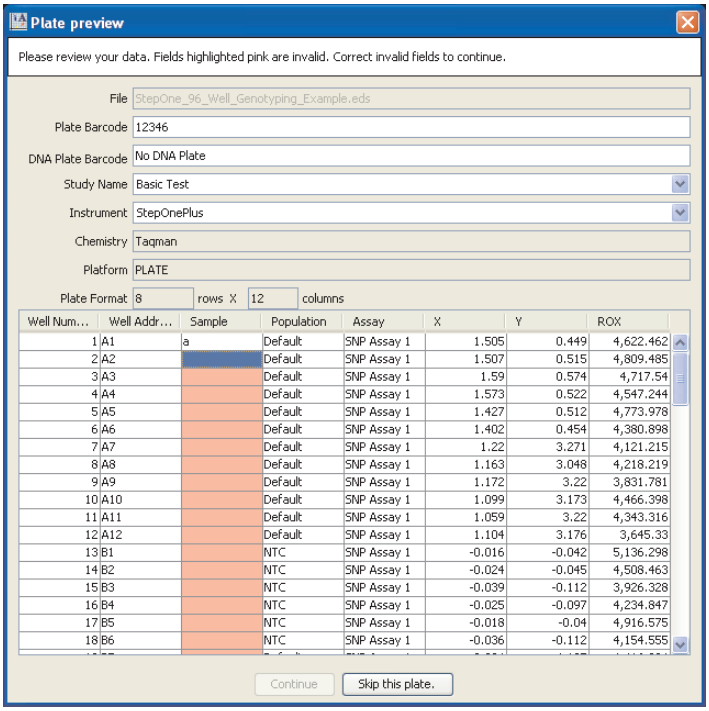
If the EDS file is missing information, the Plate Preview dialog box opens. Any field with missing information is shown in pink.

Notes

6. Click in the pink fields and enter the missing information. You can also use the right-click commands **Fill Down**, **Copy**, **Paste**, and **Delete**. When all fields are complete, click **Continue**.

   or

   Click **Skip This Plate** to omit the plate.



Well Num...	Well Addr...	Sample	Population	Assay	X	Y	ROX
1	A1	a	Default	SNP Assay 1	1.505	0.449	4,622.462
2	A2		Default	SNP Assay 1	1.507	0.515	4,809.485
3	A3		Default	SNP Assay 1	1.59	0.574	4,717.54
4	A4		Default	SNP Assay 1	1.573	0.522	4,547.244
5	A5		Default	SNP Assay 1	1.427	0.512	4,773.978
6	A6		Default	SNP Assay 1	1.402	0.454	4,380.898
7	A7		Default	SNP Assay 1	1.22	3.271	4,121.215
8	A8		Default	SNP Assay 1	1.163	3.048	4,218.219
9	A9		Default	SNP Assay 1	1.172	3.22	3,831.781
10	A10		Default	SNP Assay 1	1.099	3.173	4,466.398
11	A11		Default	SNP Assay 1	1.059	3.22	4,343.316
12	A12		Default	SNP Assay 1	1.104	3.176	3,645.33
13	B1		NTC	SNP Assay 1	-0.016	-0.042	5,136.298
14	B2		NTC	SNP Assay 1	-0.024	-0.045	4,508.463
15	B3		NTC	SNP Assay 1	-0.039	-0.112	3,926.328
16	B4		NTC	SNP Assay 1	-0.025	-0.097	4,234.847
17	B5		NTC	SNP Assay 1	-0.018	-0.04	4,916.575
18	B6		NTC	SNP Assay 1	-0.036	-0.112	4,154.555

7. When the File Progress field in the Loading dialog box displays *Done*:

   a. Review the information for each assay:

Column	Description
Assay Name	The assay name or ID number.
Plate	The name or barcode of the reaction plate in which the assay was run. The name or barcode is entered by the user in the file (page 7).
DNA Plate	The name or barcode of the source DNA plate that was used to seed the reaction plate: • *<DNA plate name>* – The software displays the source DNA plate name if the user included the name in the file (page 7) or in the sample set file (page 105). • *No DNA Plate* – The software displays *No DNA Plate* for any reaction plates that do not have a name or multiplate assigned.
Wells	The number of wells in the reaction plate containing sample/assay.
Status	SUCCESS or ERROR
Assay Codes	The Auto-Code(s) automatically or manually assigned to an assay, as defined by the analysis criteria. For a list of Auto-Codes and the corresponding analysis criteria, see page 22.

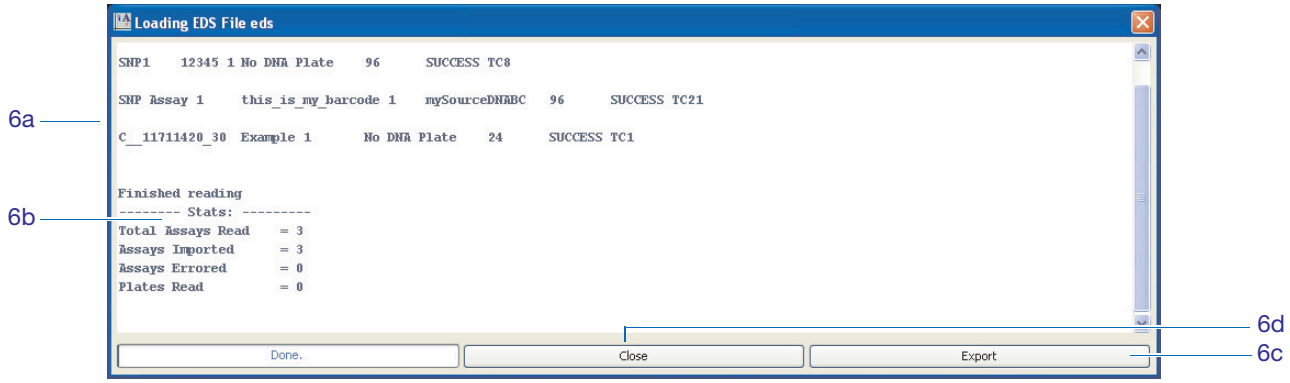
Notes

b. Review the statistics information (Stats):

- If the Assays Errored field displays 0, the EDS file(s) were imported successfully.
- If the Assays Errored field displays >0, some or all of the EDS file(s) were not imported. See "Importing errors" on page 32.

c. (*Optional*) Click **Export** to export and archive the information in the Loading dialog box.

d. Click **Close**.

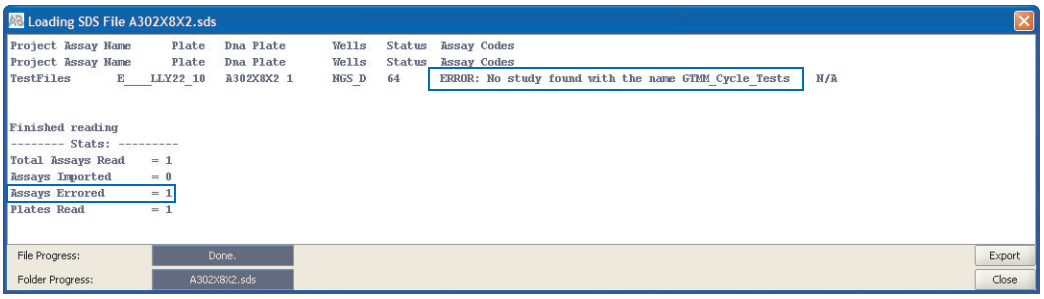


On import, the AutoCaller software automatically analyzes the data in the EDS files. To search for assays and view/edit the data, see Chapter 4 on page 55.

Importing errors

If the AutoCaller software did not successfully import a file, the Loading dialog box includes the following information:

- The Status column displays *ERROR*, along with a description of the error.
- The Assays Errored field displays the number of assays with importing errors.



The following errors may occur during import:

Error	Cause
Errors for All Types of Files	
No study found with the name *<study name>*	You selected the **Use study indicated in the file** option, but the study does not exist in the AutoCaller software.

Notes

Error	Cause
Duplicate Plate	You selected a file (plate) that was already imported into the study. **Note:** If you want to import a duplicate file (plate) into a study, see "REDO" on page 70.
Unknown sample	If you selected a sample set other than **Generic Sample Set**, a sample is included in the file that is not in the sample set list.
Errors Specific to SDS Files	
<*study name*> not found in plate comments	You selected the **Use study indicated in the file** option (step 3 on page 24), but the SDS file does not include a study name.
No plate barcode specified for <*SDS file*>.	The SDS file does not include the reaction plate barcode. For more information, see "Set up an SDS file" on page 7.

Notes

Manage studies

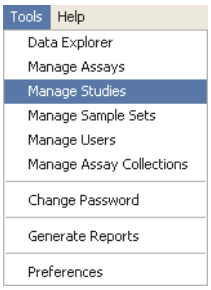
The Study Management window displays a list of all studies in the AutoCaller software database. You can use the Study Management window to:

- Add a study (as described in "Create studies" on page 19).
- View study information (below).
- Edit a study:
  - Change basic study information (page 35).
  - Change analysis criteria (page 37).
  - Reassign genotypes for multiplate assays with NEW status (page 39).
- Export a study in *.xml format (as described in Appendix B).

View study information

View the Study Management window to determine if all the expected data was imported into a study.

1. From the main window toolbar, select **Tools ▸ Manage Studies**.



2. In the Study Management window, check the study information for accuracy. The Study Management window includes the following information:

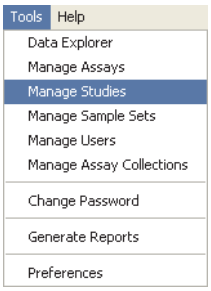
Column	Description
Name	The name of the study.
Date Created	The date the study was created.
Sample Set	The name of the sample set selected when the study was created. If a sample set was not selected, Generic Sample Set is displayed.
Platform	The consumable used in the experiment: • **Plate** indicates a 96- or 384-well reaction plate. • **Open Array** indicates a TaqMan® OpenArray™ Genotyping Plate.
# Assays	The number of assays present in the study.
Comment	Comments entered when the study was created.

Notes

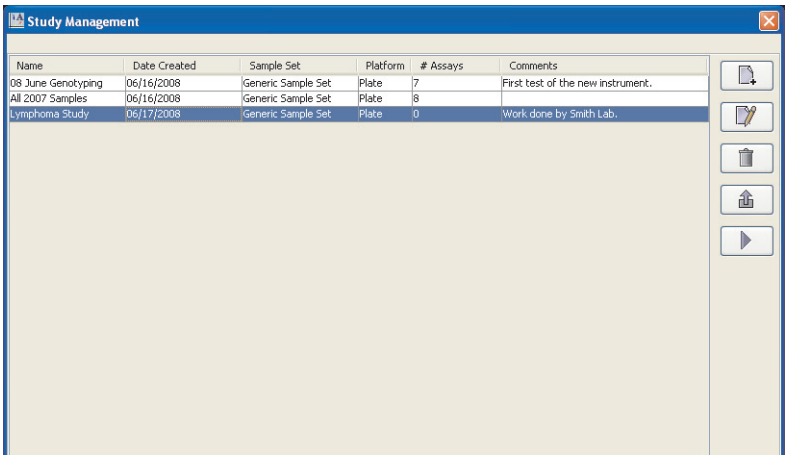
Edit a study

Change basic study information

1. From the main window toolbar, select **Tools ▸ Manage Studies**.



2. In the Study Management window:
   a. Select the study you want to edit.
   b. Click **Edit an existing study**.



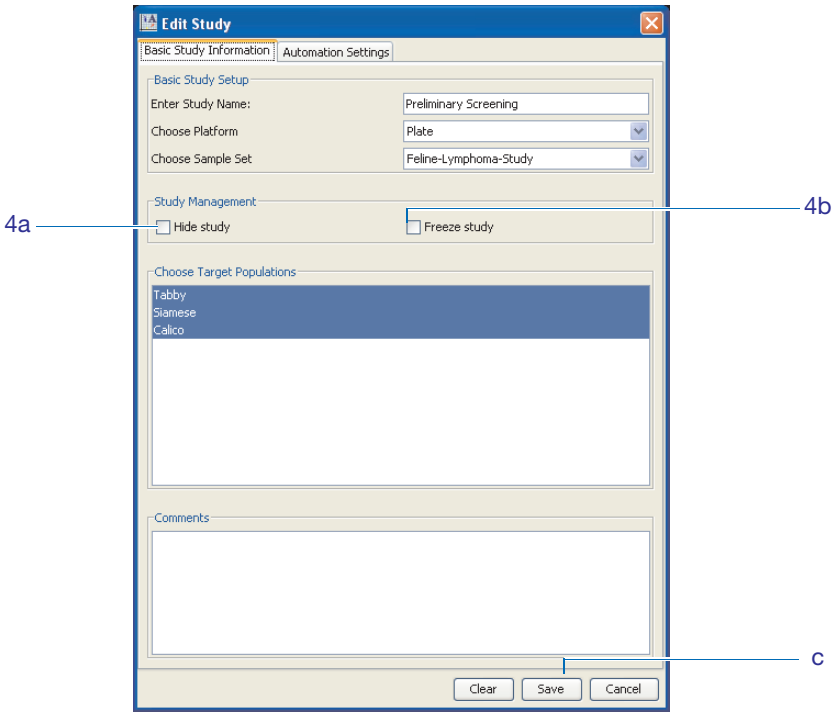
3. In the Edit Study dialog box, select the **Basic Study Information** tab (default).
4. Edit the information. You can:
   a. Select/deselect the **Hide study** check box. When you hide a study, the study will not appear in any of the software drop-down menus. (For example, the study will not appear in the Choose Study drop-down menu when you search for assays or select report criteria.)
   b. Select/deselect the **Freeze study** check box. When you freeze a study, the study cannot be edited.

> **Note:** Users at the Administrator and Scientist User Levels can unfreeze any study at any time.

Notes

c. If a study does not include data (that is, an SDS file has not yet been imported into the study), you can also edit the study name, platform, sample set, and population.

IMPORTANT! If a study includes data, you cannot edit the study name, platform, sample set, or population.



5. Save your changes. Click **Save,** then click **OK** at the prompts.

6. Click **Close** to close the Study Management window.

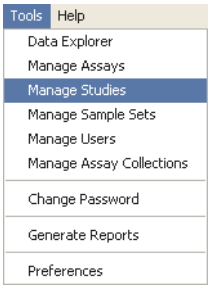
Notes

Change analysis criteria

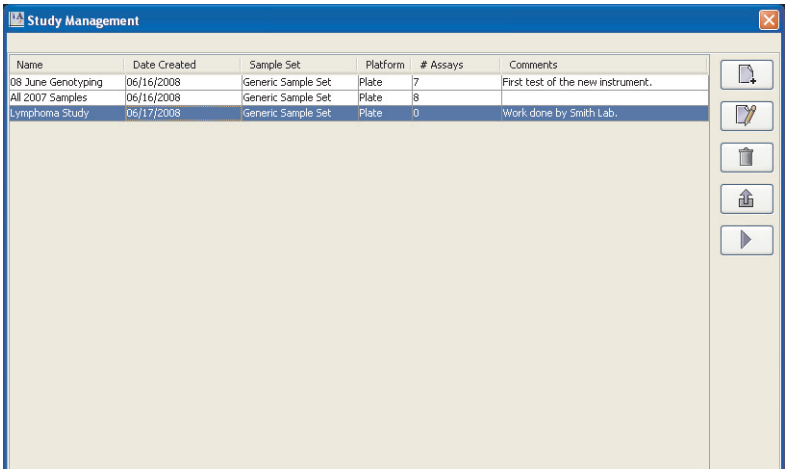
IMPORTANT! If you change the analysis criteria for a study, the software applies the new criteria only to new files imported into the study. The analysis criteria are not changed for data already in the study.

IMPORTANT! Analysis criteria are study-specific. The criteria are applied only to the current study and are not applied to any other studies in the AutoCaller software database.

1. From the main window toolbar, select **Tools** ▸ **Manage Studies**.



2. In the Study Management window:
   a. Select the study to edit.
   b. Click **Edit an existing study**.



3. In the Edit Study dialog box, select the **Automation Settings** tab.

Notes

4. Edit the information. You can:

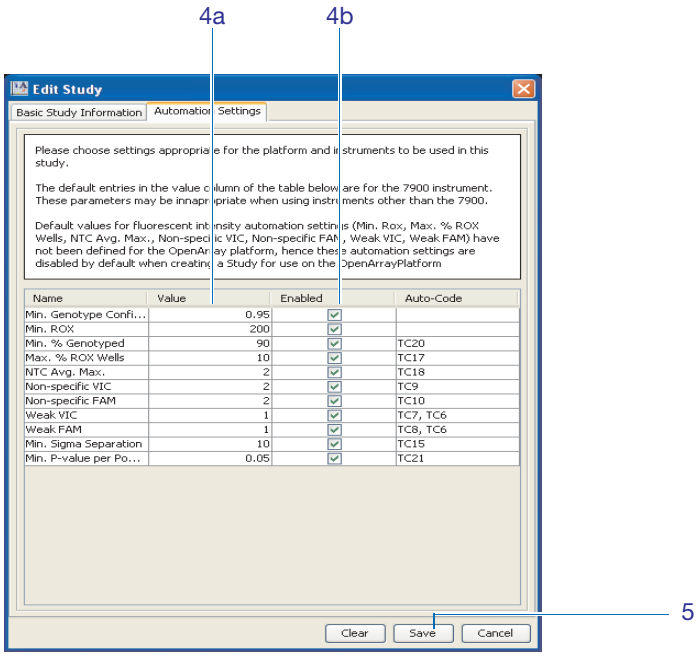
   a. Change a value for a criterion.

   Note: Applied Biosystems recommends accepting the default values for the analysis criteria.

   b. Enable/Disable the analysis criteria. When you enable an analysis criterion, the AutoCaller software applies the criterion to the study. When you disable an analysis criterion, the software ignores it.

Note: The types of analysis criteria (Name column) and the Auto-Codes are predefined in the AutoCaller software and cannot be edited. For more information, see "About the automation settings" on page 22.

5. Click **Save**.



6. At the prompts, click **OK** to save your changes to the study.

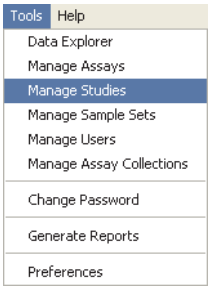
7. Click **Close** to close the Study Management window.

Notes

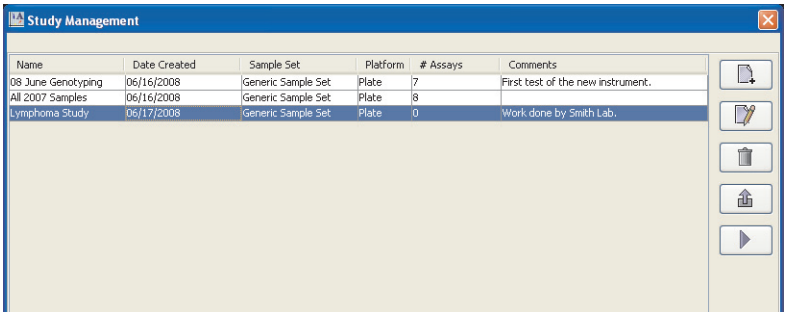
Reassign genotypes for multiplate assays with NEW status

You can assign genotypes for all multiplate assays in a study that have the status NEW. Regenotyping also reassigns the genotypes in the individual assays.

1. From the main window toolbar, select **Tools ▸ Manage Studies**.

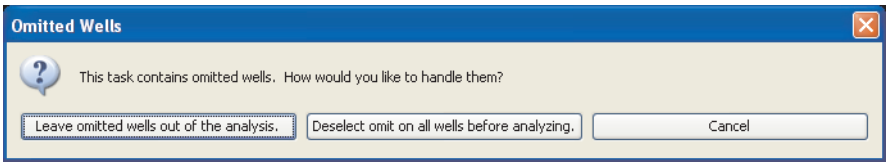


2. In the Study Management window:
   a. Select the study for which you want to reassign genotypes.
   b. Click [▶] **Re-genotype multiplates in selected study**.



   c. At the prompt, click **Yes**.

3. If you have omitted wells for any of the assays in this study, the Omitted Wells dialog box opens.



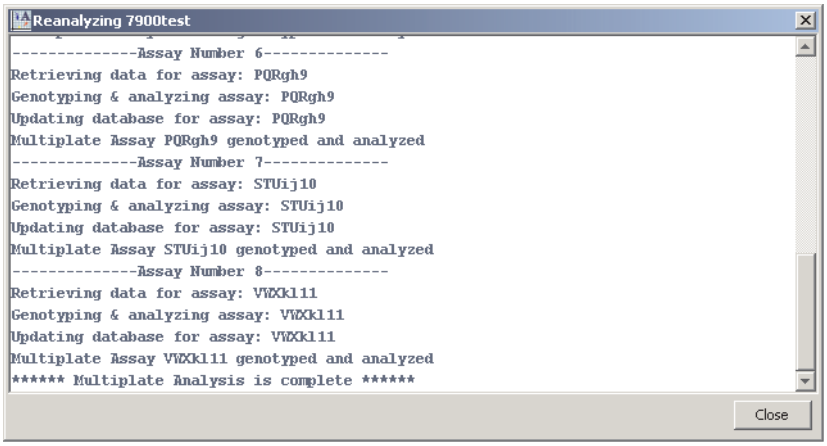
   - To continue to omit the wells in the analysis, click **Leave omitted wells out of the analysis**. The omitted wells retain their omitted status and are not re-genotyped.

     or

   - To include all wells in the analysis, click **Deselect omit on all wells before analyzing**. The status of the omitted wells changes to "included" and they are re-genotyped.

Notes

The Reanalyzing *Study_Name* window opens and displays the progress of the re-genotyping.



4. When the analysis is complete, click **Close**.

Note: If you have changed the analysis criteria for a study (page 37), the software applies the new criteria to all the multiplates in the study as part of the re-genotyping.

5. Click **Close** to close the Study Management window.

Notes

Edit assay information

About editing assay information

When you import an SDS, EDS, or OpenArray file into the AutoCaller software database, all of the assay names and ID numbers in the file are automatically added to the Assay Management window. You can then manually add or edit basic information for each assay in the Assay Management window. The workflow for adding information to an assay:

1. Create and import a text file that contains basic information for the assays (below).

   or

   Edit each assay individually in the Assay Management window ("View or edit assay information" on page 45). This may be more time-consuming.

2. Use the Assay Management window to:
   - View and edit assay information (page 45).
   - Clear assay information (page 45).
   - Delete an assay from the AutoCaller software database (page 47).

Note: The Assay Management window allows you to manage basic information about any assay in the AutoCaller software database (for example, you can change chromosome or allele assignments). The Assay Management window does *not* allow you to search for assays or view/edit run data. To perform these functions, see Chapter 4 on page 55.

Import assay information from a text file

Overview

There are two types of assay information files that you can import into the AutoCaller software database: a data file (in SDS, EDS or OpenArray formats) or a text (*.txt) file.

- When you import a data file, the AutoCaller software imports assay names or ID numbers that is shown in the Assay Management window *and* all run data associated with the assays (see "Import files" on page 24).
- When you import a text file (*.txt), the AutoCaller software imports basic information about assays that is shown in the Assay Management window, but it does *not* import any run data.

  Import a text file to:

  – Add new information (to be shown in the Assay Management window) for an assay.

  – Update existing information (to be shown in the Assay Management window) for an assay.

Instructions for creating and importing a text file start on page 42.

Notes

Create the text file

Create a text file (*.txt) to add basic information for many assays to the AutoCaller software database at the same time.

1. Open a spreadsheet program (such as Microsoft® Excel software).
2. On line one, enter the following column headings:

Column Heading	Description of Column Contents
Assay ID	The assay name or ID number, as it appears in the SDS file.
Chromosome	The chromosome assigned to the assay.
Priority Code	The priority code assigned to the assay. Priority codes, defined by the user, can be used to facilitate searches. For example, you may want to use the gene name as a priority code.
Actual Allele 1	The actual alleles present in the sample.
Actual Allele 2	
Display Allele 1	The alleles you want to display in your published reports. By default, reports use the actual allele names. Enter alternative names for the alleles in the Display Allele columns. For example, you might use A/T in the Actual Allele 1 and 2 columns, but +/- in the Display Allele 1 and 2 columns.
Display Allele 2	
Comment	Comments about the assay to add to the database.

3. On the following lines, enter information for each assay. You can list the assays in any order, but you must follow these parameters:
   - Enter one assay per line.
   - Enter the assay name or ID number exactly as it appears in the file.

     IMPORTANT! When you import a file into the AutoCaller software database, all of the assay name or ID numbers that are in the file are added to the Assay Management window. To ensure that any assay information you import into the Assay Management window is associated with the correct assay, enter the assay name or ID number exactly as it appears in the file.

   - (*Optional*) Enter the remaining information listed in step 2 above.

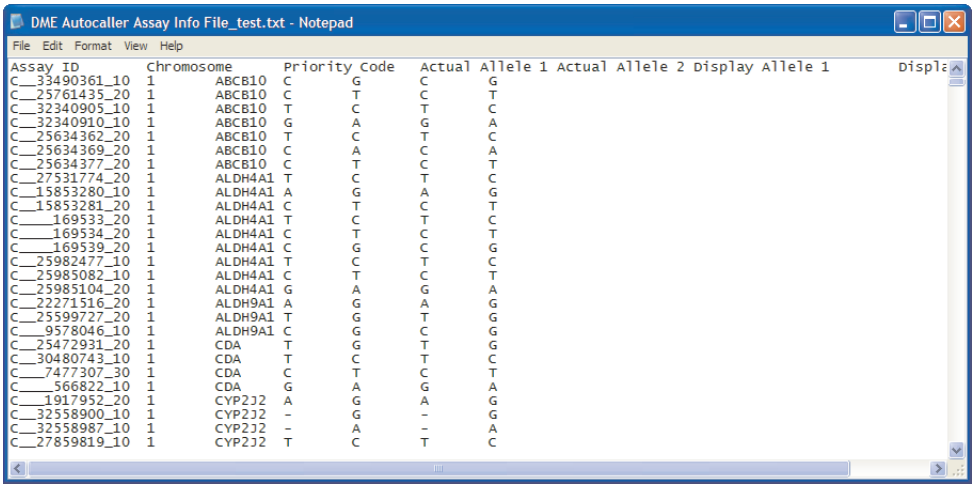
2

3

	A	B	C	D	E	F	G
1	Assay ID	Chromosome	Priority Code	Actual Allele 1	Actual Allele 2	Display Allele 1	Display Allele 2
2	C__33490361_10	1	ABCB10	C	G	C	G
3	C__25761435_20	1	ABCB10	C	T	C	T
4	C__32340905_10	1	ABCB10	T	C	T	C
5	C__32340910_10	1	ABCB10	G	A	G	A
6	C__25634362_20	1	ABCB10	T	C	T	C
7	C__25634369_20	1	ABCB10	C	A	C	A
8	C__25634377_20	1	ABCB10	C	T	C	T
9	C__27531774_20	1	ALDH4A1	T	C	T	C
10	C__15853280_10	1	ALDH4A1	A	G	A	G
11	C__15853281_20	1	ALDH4A1	C	T	C	T
12	C____169533_20	1	ALDH4A1	T	C	T	C
13	C____169534_20	1	ALDH4A1	C	T	C	T
14	C____169539_20	1	ALDH4A1	C	G	C	G
15	C__25982477_10	1	ALDH4A1	T	C	T	C
16	C__25985082_10	1	ALDH4A1	C	T	C	T
17	C__25985104_20	1	ALDH4A1	G	A	G	A

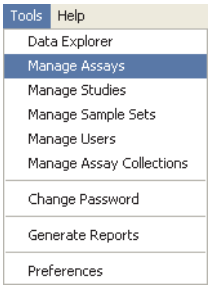
Notes

4. Save the spreadsheet as a text (*.txt) file.

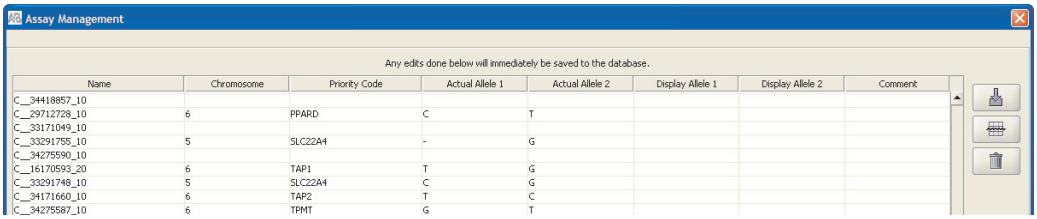


Import the text file

1. From the main window toolbar, select **Tools** ▸ **Manage Assays**.

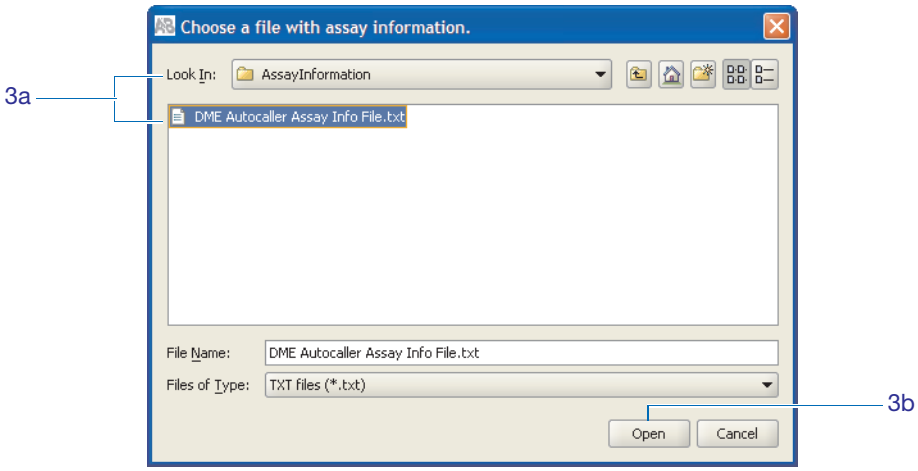


2. In the Assay Management window, click  **Load or update assay information**.



3. Select the text file. In the Choose a file with assay information dialog box:
   a. Browse to and select a file.
   b. Click **Open**. The Assay Info Load Progress dialog box appears while the assay information is being imported.

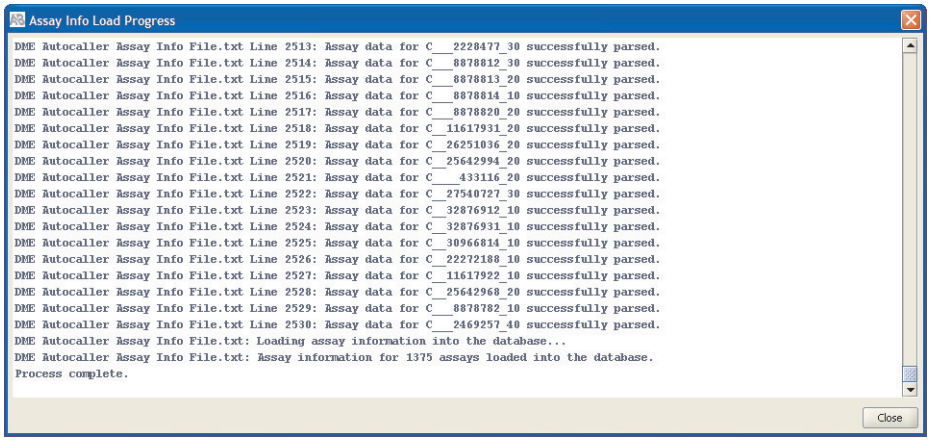
Notes



4. When the Assay Info Load Progress dialog box displays *Process complete*, review the information for each assay. Each line in the Assay Info Load Progress dialog box includes the following information:
   - Text file name
   - Line number
   - Assay status: *Assay data for <assay name> successfully parsed* or an error message. If the software displays an error message for an assay, the assay was not imported.

   Note: Line 1 always displays the error message *Invalid chromosome*, because Line 1 in the text file lists the column headings and not any assay information.

   After reviewing assay information, click **Close**. The new or updated assay information is displayed in the Assay Management window.



5. Click **Close** to close the Assay Management window.

Notes

Edit assay information in the Assay Management window

View or edit assay information

1. From the main window toolbar, select **Tools** ▸ **Manage Assays**.

2. In the Assay Management window, locate the assay of interest, then view the assay information.

3. You can edit the assay information in any column except Name. To edit assay information:

To edit a...	Do this...
Chromosome	Click the Chromosome field for an assay, then select a chromosome from the drop-down menu.
Priority Code	Double-click the Priority Code field for an assay, then enter a code. **Note:** You can enter any text in the Priority Code field.
Actual Allele 1 Actual Allele 2 Display Allele 1 Display Allele 2	Click the field for an assay, then select an option from the drop-down menu. You can select one of the following: • The base for the SNP – **A**, **C**, **G**, or **T**. • Plus sign (**+**) – Indicates the presence of a mutation or indicates an insertion. • Minus sign (**-**) – Indicates the absence of a mutation or indicates a deletion.
Comment	Double-click the Comments field, then enter comments.

Clear assay information from the Assay Management window

This feature clears assay information from the Assay Management window only. It does not delete the assay or any run data for the assay from the AutoCaller software database. (To delete an assay from the database, see page 51.)

1. From the main window toolbar, select **Tools** ▸ **Manage Assays**.

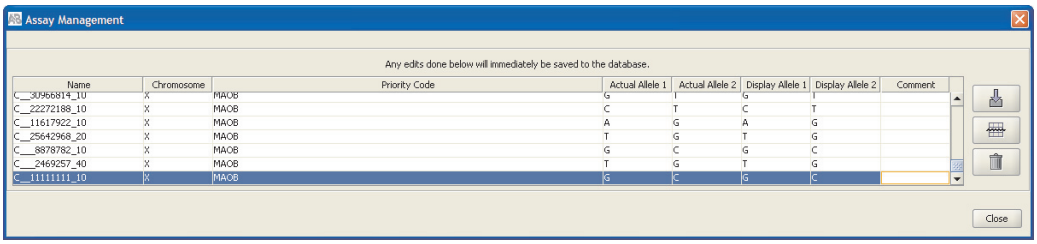
Notes

2. Select the assay to clear. In the Assay Management window:

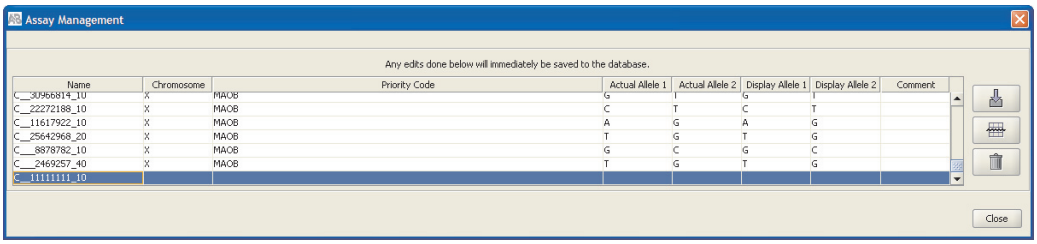
   a. Select the assay(s) for which you want to clear information.

   Note: Press **Ctrl+click** or **Shift+click** to select multiple assays.

   b. Click  **Clear data for the selected assays**.



3. Confirm that the information has been cleared for the selected assay.

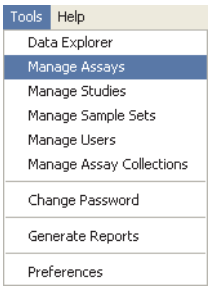


Notes

Delete an assay

You can delete an assay which does not have run data associated with it. To delete assays which area associated with run data, see page 52.

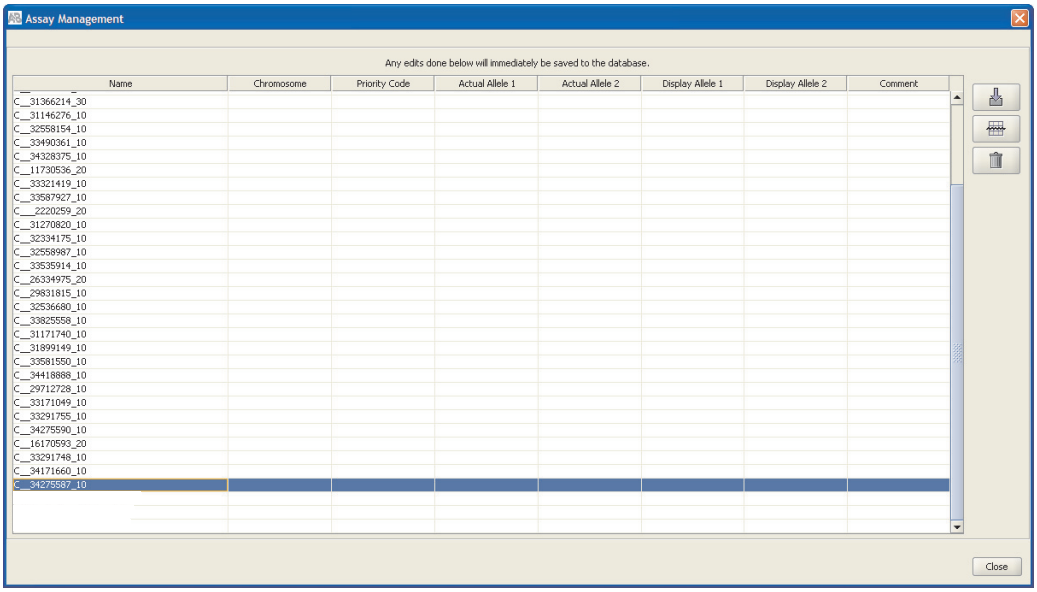
1. From the main window toolbar, select **Tools** ▸ **Manage Assays**.



2. In the Assay Management window:
   a. Select the assay(s) you want to delete.

   Note: Press **Ctrl+click** or **Shift+click** to select multiple assays.

   b. Click [🗑] **Delete the selected assays**.



3. At the prompt, click **OK** to delete the assay(s) from the AutoCaller software database.

   Note: If a study has run data associated with the assay, the software displays an error message and does not delete the assay. You can delete assays and associated run data in the Data Explorer window (see page 51).

4. Click **Close** to close the Assay Management window.

Notes

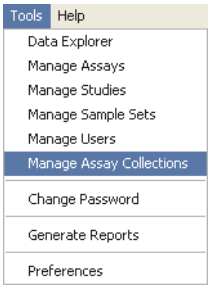
Manage assay collections

Create an assay collection when you want to search for more than one assay at a time in the Search dialog box. An *assay collection* is a group of two or more assays.

Create an assay collection

You can create an assay collection by choosing the assays one by one or by importing an assay collection from a text file (*.txt). The file should contain each assay name on a separate line.

1. Select **Tools** ▸ **Manage Assay Collections**.



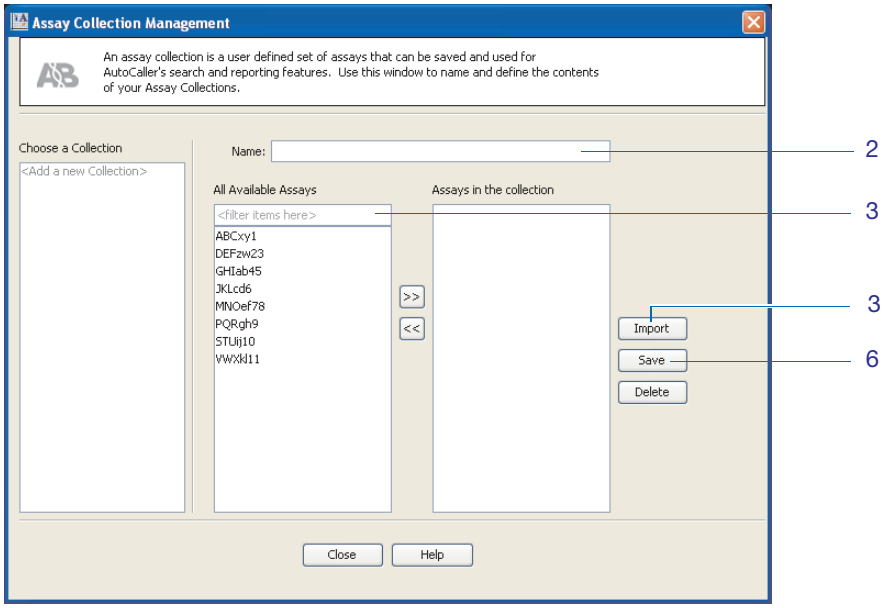
2. Name the new assay collection. In the Manage Assay Collections dialog box, enter a name in **Name** field. Assay collection names can contain only letters, numbers, underscores, and spaces.

Notes

3. Select assays for the collection. Either:
   - Click the name of the assay in the All Available Assays list and then click >>. Repeat for all assays to be added to the collection.

     (Optional) To quickly find an assay, enter the first few letters of the assay name in the **<filter items here>** field above the All Available Assays list. The list scrolls to display assays that begin with the letters you entered.

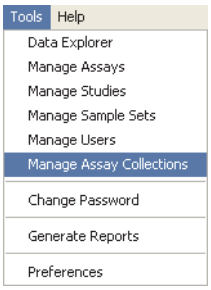
     or
   - Click **Import**. In the dialog box that opens, locate the file containing the assay collection and click **Open**.



4. Verify that the assays in the Assays in the Collection list are correct.
5. If needed, follow the instructions in "Edit an assay collection" to add or remove assays.
6. Click **Save** to save the assay collection, then **Close** to close the dialog box. At the prompt, click **Yes**.

Edit an assay collection

1. Select **Tools ▸ Manage Assay Collections**.



2. In the Choose a Collection list, click the name of the collection to be edited.

Notes

3. To add an assay to the collection, click the name of the assay in the All Available Assays list and then click **>>.**

   To quickly find an assay, enter the first few letters of the assay name in the **<filter items here>** field above the All Available Assays list. The list scrolls to display assays that begin with the letters you entered.

4. To add assays to the collection from a text file (*.txt):

   a. In the Assay Collection Management dialog box, click **Import**.

   b. In the dialog box that opens, locate the file containing the assay collection and click **Open**.

      The file should contain each assay name on a separate line.

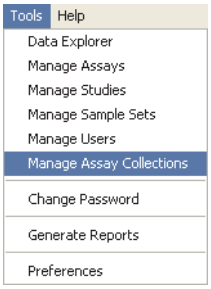
   c. Verify that the assays in the Assays in the Collection list are correct.

5. To remove an assay from the collection, click the name of the assay in the Assays in the Collection list and then click **<<.**

6. Click **Save** to save the assay collection, then **Close** to close the dialog box. At the prompt, click **Yes**.

Delete an assay collection

1. Select **Tools ▸ Manage Assay Collections**.



2. Select the collection to delete. In the Choose a Collection list:

   a. Click the name of the assay collection to be deleted,

   b. Click **Delete**.

3. At the prompt, click **Yes** to delete the assay collection, then **Close** to close the dialog box. At the prompt, click **Yes**.

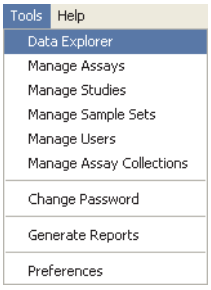
Notes

Delete assays and plates

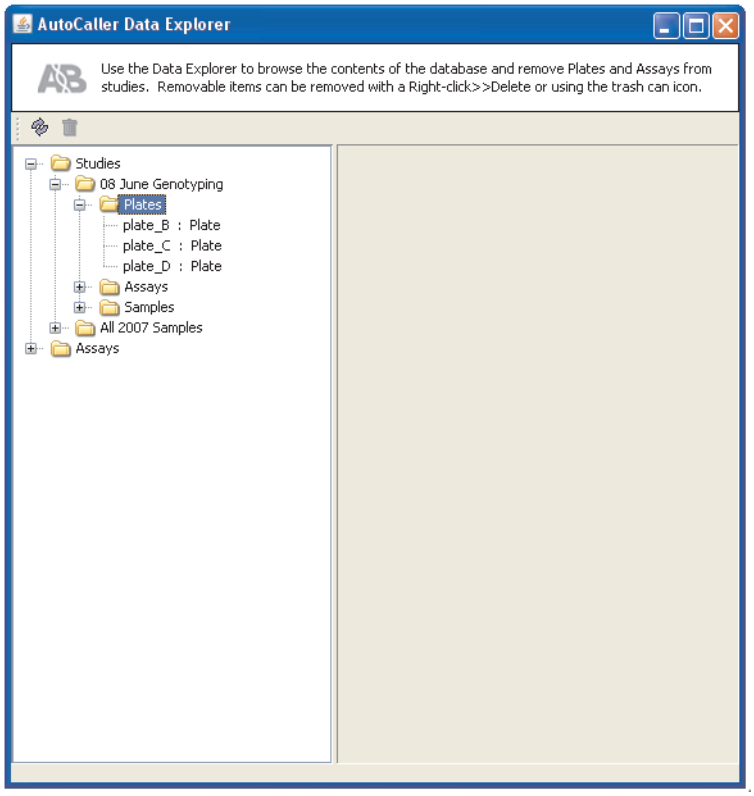
Delete an assay from a study

You can delete an assay from a study even if there is data associated with the assay.

1. Select **Tools ▸ Data Explorer**.



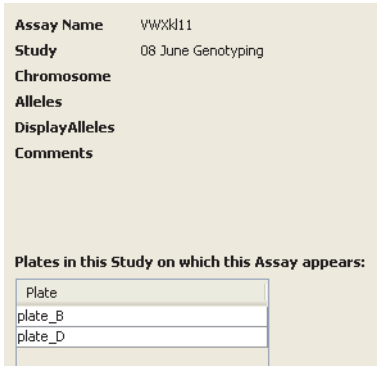
2. In the Data Explorer window, click + to open a study and view the associated plates and assays.



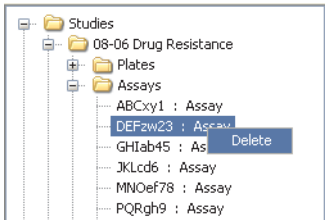
Notes

3. Delete the assay:

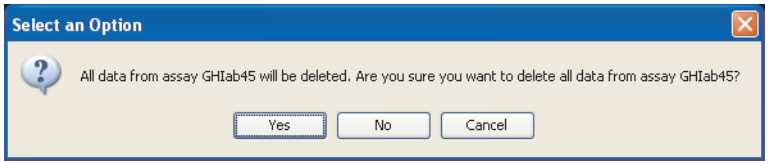
   a. Click the assay to be deleted. The right side of the Data Explorer window displays the plates associated with the assay.



   b. Right-click and select **Delete**. (You can also click (Delete).)



   c. At the prompt, click **Yes** to delete the assay from the study. Any results for runs containing this assay are deleted from the AutoCaller software database.

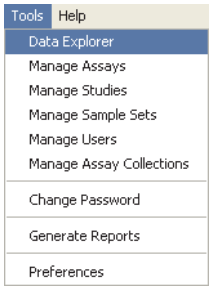


4. Click **OK** in the message box, then click (Close) to close the Data Explorer window.

   The assay is deleted from the study and from the AutoCaller software database.

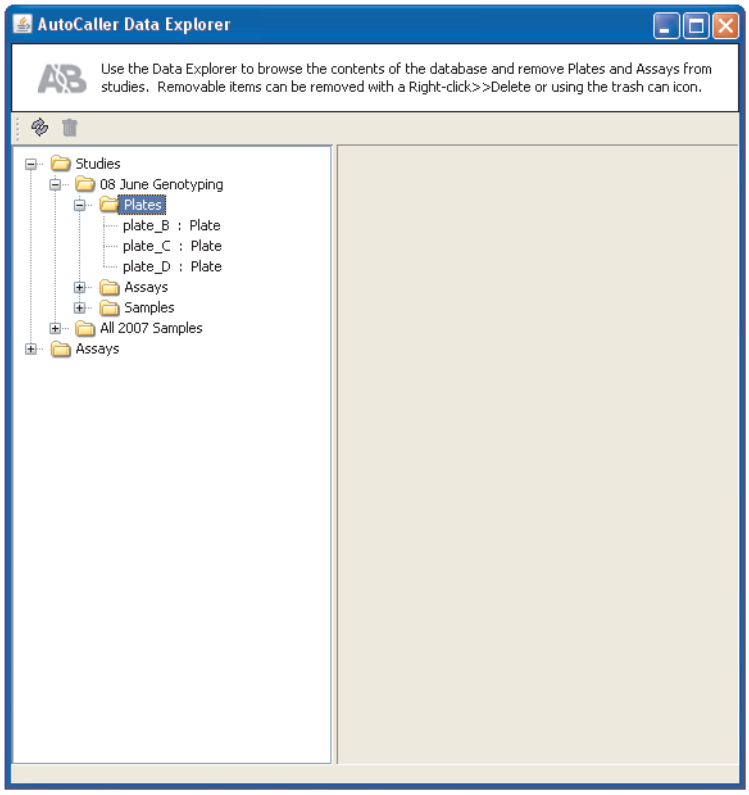
Delete a plate from a study

1. Select **Tools** ▸ **Data Explorer**.



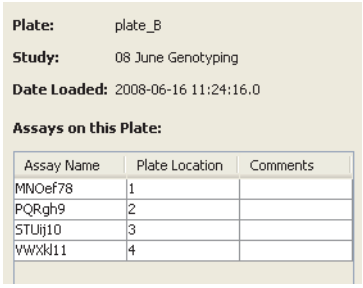
Notes

2. In the Data Explorer window, click + to open a study and view the associated plates and assays.

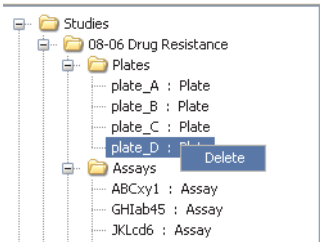


3. Delete the plate:

   a. Click the plate to be deleted. The right side of the Data Explorer window displays the assays associated with the plate.

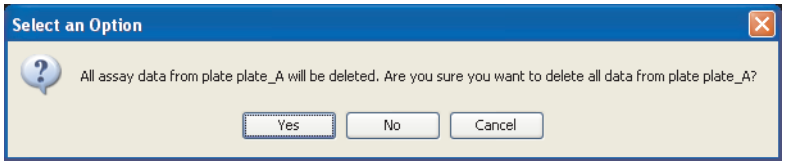


   b. Right-click and select Delete. (You can also click (Delete).)



Notes

c. At the prompt, click **Yes** to delete the plate from the study.



4. Click **OK** in the message box, then click (Close) to close the Data Explorer window.

   The plate is deleted from the study.

Notes

4 View and Edit Data

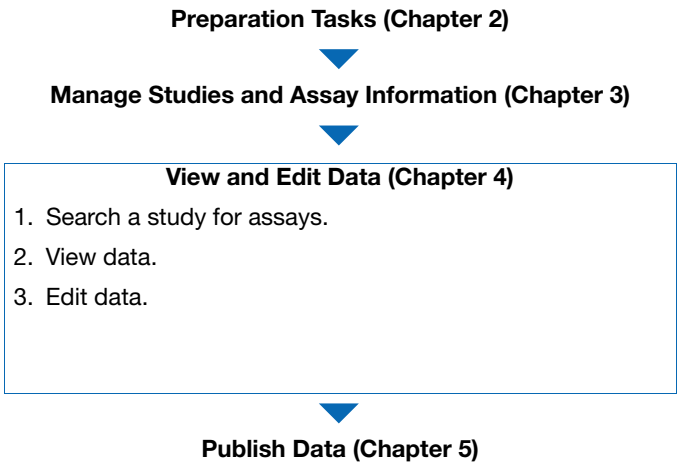
This chapter covers:

Chapter overview

This chapter explains how to search a study in the Applied Biosystems AutoCaller™ Software database for assays, then view and edit data in the studies.

IMPORTANT! Any edits you make to the data are saved to the AutoCaller software database. Your edits are tracked with your user name and the date and time you made the edits.

Workflow



Required user levels

To perform the procedures in this chapter, log in to the AutoCaller software as a member of the required User Level. The required User Level is indicated at the beginning each section in this chapter. For information on User Levels, see "About user levels" on page 3.

Notes

Search a study for assays

Required user level

To perform the procedures in this section, log in to the AutoCaller software at any User Level.

About the search criteria

In the Search for Assays dialog box, you can optionally select any of the following search criteria to either narrow or broaden your search:

Criterion	Description
Assay Name	The assay name or ID number.
Assay Collection	A name given to a group of two or more assays.
Plate Barcode	The name or barcode of the reaction plate in which the assay was run.
Date Loaded	The date the assay was imported into the AutoCaller software database.
TC Code	The Auto-Code automatically or manually assigned to an assay, as defined by the analysis criterion. For a list of Auto-Codes and the corresponding analysis criteria, see page 22.
Status	The analysis status of the assay. For more information, see "Status menu" on page 70.
DNA Plate	The name or barcode of the source DNA plate (that is, the DNA plate that was used to seed the reaction plate): • *<DNA plate name>* – The software displays the source DNA plate name if the user included the name in the file (page 7) or in the sample set file (page 105). • *MULTI* – The software assigns the name *MULTI* whenever there is more than one plate in the study with the same assay. For more information, see "Select a multiplate" on page 71. • *No DNA Plate* – The software displays *No DNA Plate* for any reaction plates that do not have a name or multiplate assigned.
Priority Code	The priority code assigned by a user to the assay in the Assay Management window, used to perform searches. used to facilitate searches (for example, a gene name can be used as a priority code).
Chromosome	The chromosome assigned by a user to the assay in the Assay Management window.
Comments	Comments entered by a user from the Assay Management window or from the Comments tab o f the Analyze window.

Notes

Search for assays in a study

1. From the Analyze window toolbar, click **Search for assays** to open the Search for Assays dialog box.
2. From the Choose Study drop-down menu, select the study in which you want to search for assays.
3. (*Optional*) In the Assay Name field, enter the name or ID number of the assay to find.

   To search for more than one assay at a time, click (Open) to open a dialog box where you can enter or paste more than one assay name.

   You can enter an asterisk (*) as a wild card. For example, if you enter ***245***, the search will return all assays with names that include a 245 sequence: C__23**245**6789_20, C__33**245**6789_20, and so on.

   If you do not specify an assay name or ID number, the software returns all assays in the study.
4. *(Optional)* In the Assay Collection drop-down menu, define the assay collection:
   - Select the assay collection in which you want to search for assays.

     or
   - Select **Define a new collection** to define a new assay collection for use as a search criterion. See page 48 for instructions.
5. (*Optional*) In the Plate Barcode field, enter the barcode for the reaction plate.

Note: Entering a barcode is useful when you want to see all the assays run on a single reaction plate.

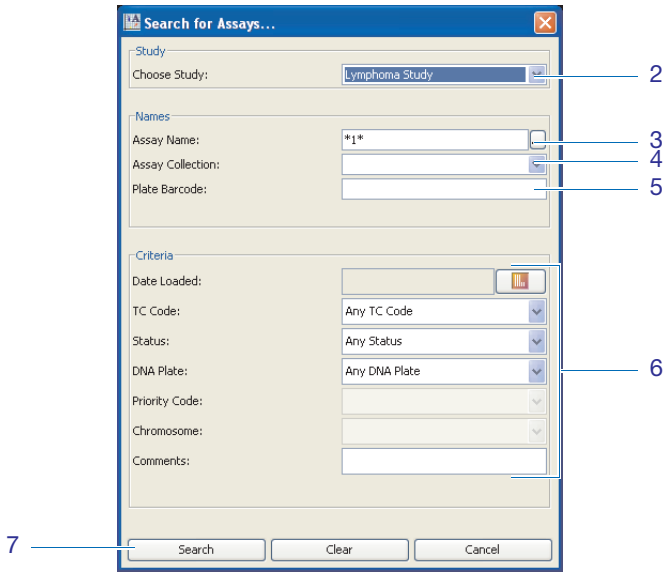
Notes

6. (*Optional*) In the Criteria pane, specify any of the following criteria to narrow the set of assays returned by the search:

Field	To specify this criterion...
Date Loaded	Click **Choose Date Assays Were Loaded**, then select the date the assay was imported into the AutoCaller software database.
TC Code	Select the Auto-Code from the drop-down menu.
Status	Select the analysis status from the drop-down menu.
DNA Plate	Select the source DNA plate name from the drop-down menu: • ***<DNA plate name>*** (user-defined) • **MULTI** • **No DNA Plate**
Priority Code	Select a priority code from the drop-down menu.
Chromosome	Select a chromosome from the drop-down menu.
Comments	Click the **Comments** field, then enter any comments that appear in the Assay Management window (page 41) or the Comments tab of the Analyze window (page 68). Enter the comments exactly as they appear in the Assay Management or Analyze window, or enter an asterisk (*) as a wild card.

Note: If you do not specify any criteria, the software returns all assays with the name or ID number you entered in step 3. If you did not enter an assay name or ID number, the software returns all assays in the study.

7. Click **Search**. The assays returned by the search are displayed in the Assay Selection pane of the Analyze window.



Notes

8. Go to "View data" on page 60 for information on viewing the data within the Analyze window.

View data

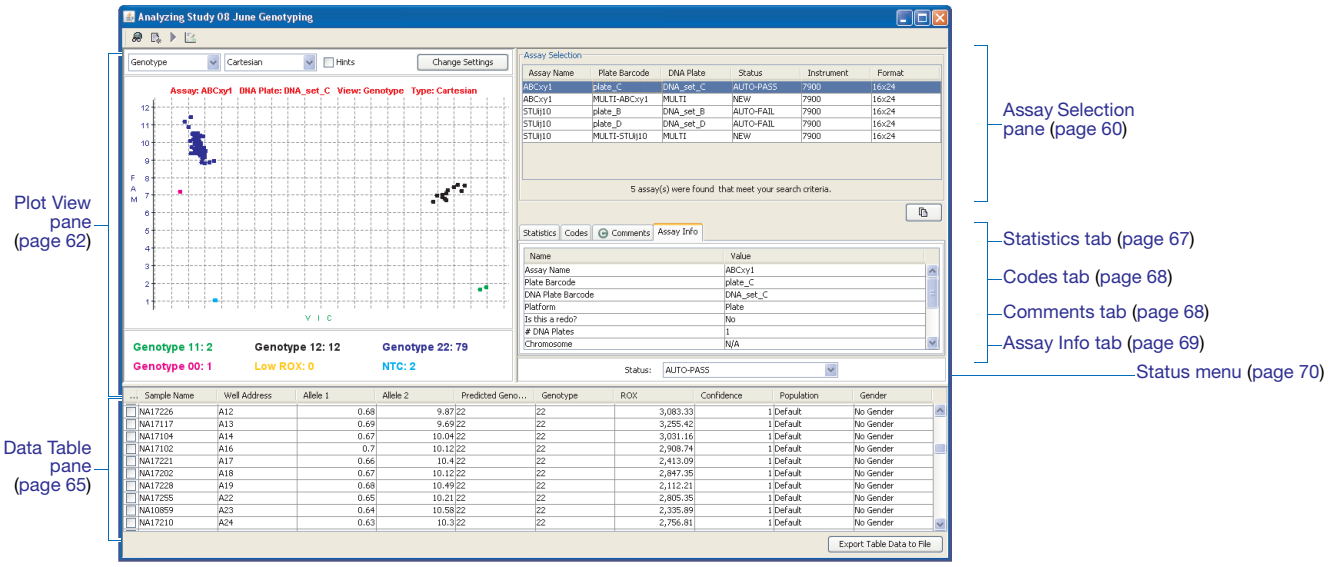
When you import an assay into the AutoCaller software database, the software automatically analyzes the data on import. This section explains how to view the analyzed data in the Analyze window. If you want to edit the data and reanalyze it, see "Edit data" on page 73.

Required user level

To perform the procedures in this section, log in to the AutoCaller software at any User Level.

Analyze Window elements

The assays returned by the search are displayed in the Analyze window. For information on using the Analyze window, see the sections cross-referenced below.



Assay Selection pane

The Assay Selection pane displays the assays returned by the search and includes the following information:

Column	Description
Assay Name	The assay name or ID number.
Plate Barcode	The name or barcode of the reaction plate in which the assay was run. The name or barcode is entered by the user in the file. The software adds *MULTI-* to the barcode (for example, *MULTI-A302GL4M)* whenever there is more than one plate in the study with the same assay. For more information, see "Select a multiplate" on page 71.

Notes

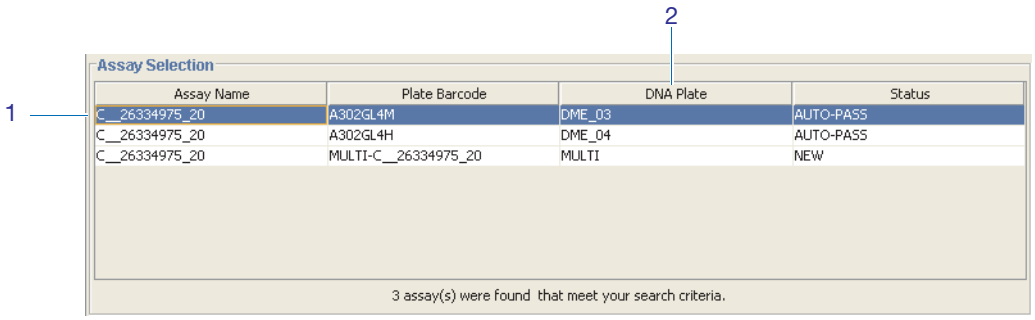
Column	Description
DNA Plate	The name or barcode of the source DNA plate (that is, the DNA plate that was used to seed the reaction plate): • *<DNA plate name>* – The software displays the source DNA plate name if the user included the name in the file or in the sample set file (page 105). • *MULTI* – The software assigns the name *MULTI* whenever there is more than one plate in the study with the same assay. • *No DNA Plate* – The software displays *No DNA Plate* for any reaction plates that do not have a name or multiplate assigned.
Status	The analysis status of the assay.
Instrument	The instrument where this assay was run.
Format	The format of the data: • *<a> rows X <b> columns* – The number of rows, *<a>*, and columns, *<b>*, in the reaction plate. • *OpenArray* – Reaction plates imported from OpenArray data.

To view data in the Assay Selection pane:

1. To select an assay, click anywhere in the assay row. The remaining panes and tabs in the Analyze window display additional information for the selected assay.

   You can select only one assay at a time.

2. To sort data, click a column heading. For example, to sort the assays by the source DNA plate name, click **DNA Plate**. The assays are sorted by plate name, in alpha-numerical order.



To change the analysis status of the assay, see "Edit data" on page 73.

IMPORTANT! You must belong to the Administrator or Scientist User Level to change the assay status.

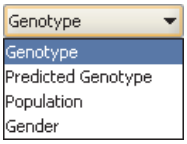
Notes

Plot View pane

The Plot View pane displays a plot of the selected assay. Each sample (point) in the plot is color-coded by the assigned genotype, as shown in the legend located beneath the plot. You can print the Plot View pane.

To view data in the Plot View pane:

- To change the data displayed in the plot, select a data type from the drop-down menu:

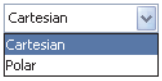


  - **Genotype** – Displays data by genotype. If a user has not edited the genotypes, the software displays the predicted genotypes. If the genotypes have been edited, the software displays the edited genotypes.

    You can edit the genotypes in three ways: manually (page 73), by clicking (Assign Genotypes) to reassign genotypes after omitting wells (page 76) in the Analyze window or, for multiplates, by clicking the (Re-genotype multiplates in selected study) in the Study Management window (page 39).
  - **Predicted Genotype** – Displays data by genotype, as automatically assigned by the AutoCaller software when the file is imported.
  - **Population** – Displays data by population. This option is only available if population information was included in the sample set file *and* a target population was selected when the study was created (step 3d on page 20).
  - **Gender** – Displays data by gender. This option is only available if gender information was included in the sample set file.

    IMPORTANT! Sample set files contain information about each sample in a reaction plate. For more information on sample set files, see Appendix A on page 103.

- To change the coordinates used to display the plot, select the coordinates from the drop-down menu:

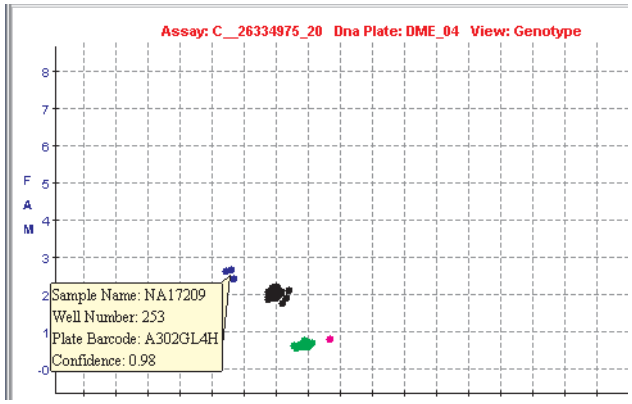


  - **Cartesian** – A plot of VIC® dye Rn versus FAM™ dye Rn, in Cartesian coordinates.
  - **Polar** – A plot of VIC dye Rn versus FAM dye Rn, in polar coordinates.

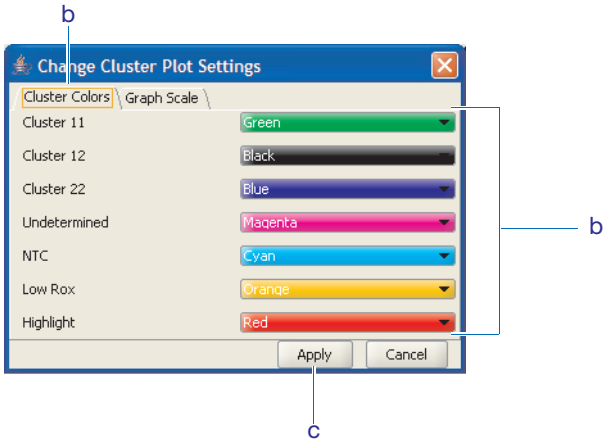
Notes

- To display hints, check **Hints** check box.

When the Hints check box is selected, the software displays information for each sample (point) in the plot when you move your mouse over the point. This is useful when you want to see if the same DNA is an outlier in numerous assays.



- To change the color of the data displayed:
  a. Click **Change Settings** to open the Change Cluster Plot Settings dialog box.
  b. Click the **Cluster Colors** tab (default), then select the desired colors from the drop-down menus.
  c. Click **Apply** to save your changes.



- To change the scale of the data displayed:
  a. Click **Change Settings** to open the Change Cluster Plot Settings dialog box.
  b. Click the **Graph Scale** tab.
  c. Select one of the following:
     - **Automatic** – Automatically scales the plot to the data.

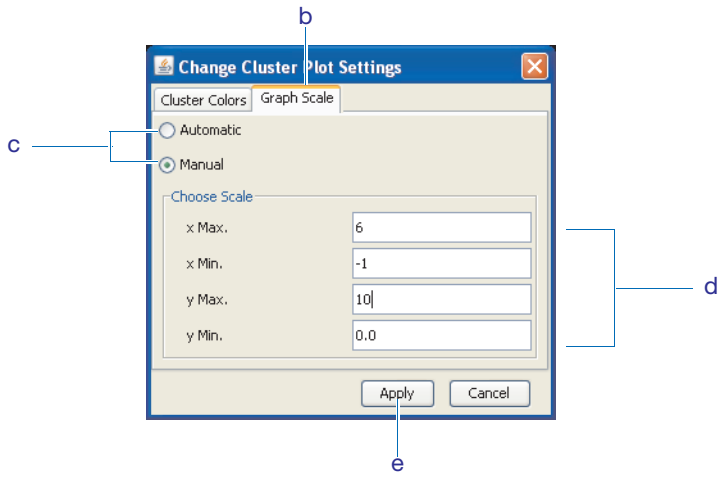
Notes

- **Manual** – Allows you to define the minimum and maximum values for the x and y axes.

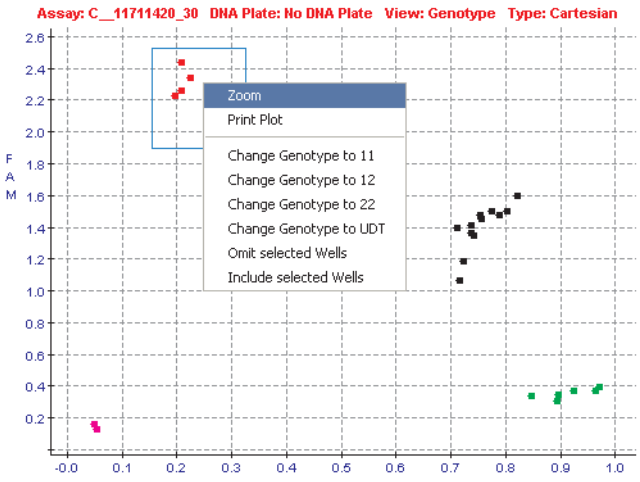
d. If you selected **Manual**, enter the desired values for the x and y axes.

Note: If data are outside the range you enter, the data does not appear in the plot. Applied Biosystems recommends the following values: x Max. = 6; x Min. = -1; y Max. = 10 or 12; y Min. = 0.

e. Click **Apply** to save your changes.



- To zoom in/out:
  - To zoom in, draw a box around the data you want to see more closely, right-click in the box, then select **Zoom** from the pop-up menu.



  - To return to the original view, double-click anywhere in the plot.
- To print the Plot View pane:

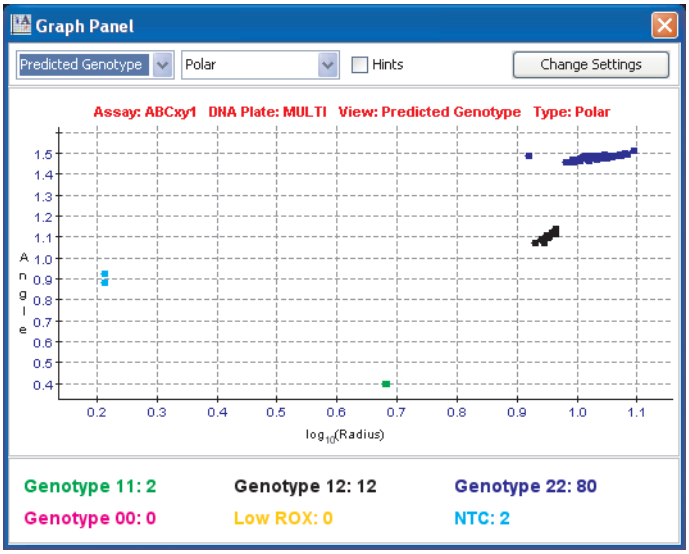
a. Right-click in the plot and select **Print Plot**.

Notes

b. When prompted, click **OK** in the Print dialog box.

- To view a second copy of the Plot View pane, click (Open New Graph Panel).

  A Graph Panel window appears, displaying the same plot as shown in the Plot View pane. You can change the plot controls as needed to view different information.



Data Table pane

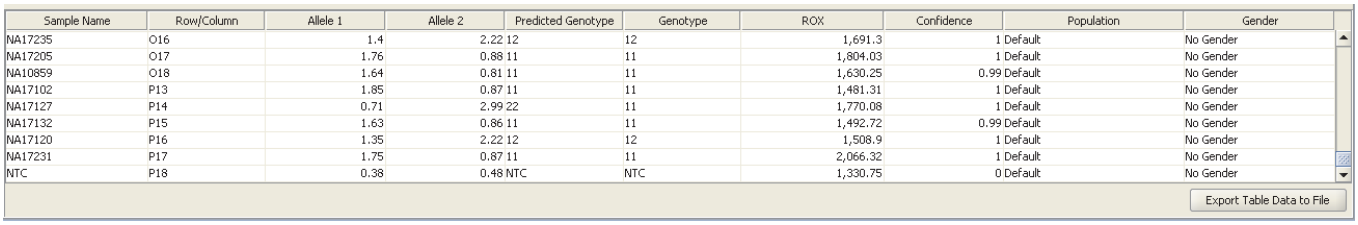
The Data Table pane displays the following information for each sample in the selected assay:

Column	Description
... (Omitted)	When checked, indicates the well is omitted from the calculations for this assay.
Sample Name	The name of the sample.
Well Address	The location of the sample in the reaction plate. For example, P18 indicates that the sample is located in row P, column 18.
Allele 1	Rn (normalized reporter) value of the VIC® dye signal.
Allele 2	Rn (normalized reporter) value of the FAM™ dye signal.
Predicted Genotype	The genotype automatically assigned by the AutoCaller software.
Genotype	The genotype edited by a user. **Note:** If the genotype is not edited by a user, the predicted genotype is displayed in this column.
ROX	The ROX™ dye level in the well. **Note:** ROX dye is in the TaqMan® master mix used in TaqMan® genotyping assays.
Confidence	The confidence value for the sample (point). The confidence value indicates the likelihood that the point belongs to the cluster (the cluster is defined by the software).

Notes

Column	Description
Population	Population information, if population information was included in your sample set file‡. If no population information was included, *Default* is displayed in the sample's Population field.
Gender	Gender information, if gender information was included in your sample set file.‡ If no gender information was included, *No Gender* is displayed in the sample's Gender field.

‡ To use sample sets, you must first create sample set files, then import the files into the AutoCaller software database. Sample set files contain information about each sample in a reaction plate. For more information on sample set files, see Appendix A on page 103.



Sample Name	Row/Column	Allele 1	Allele 2	Predicted Genotype	Genotype	ROX	Confidence	Population	Gender
NA17235	O16	1.4	2.22	12	12	1,691.3	1	Default	No Gender
NA17205	O17	1.76	0.88	11	11	1,804.03	1	Default	No Gender
NA10859	O18	1.64	0.81	11	11	1,630.25	0.99	Default	No Gender
NA17102	P13	1.85	0.87	11	11	1,481.31	1	Default	No Gender
NA17127	P14	0.71	2.99	22	11	1,770.08	1	Default	No Gender
NA17132	P15	1.63	0.86	11	11	1,492.72	0.99	Default	No Gender
NA17120	P16	1.35	2.22	12	12	1,508.9	1	Default	No Gender
NA17231	P17	1.75	0.87	11	11	2,066.32	1	Default	No Gender
NTC	P18	0.38	0.48	NTC	NTC	1,330.75	0	Default	No Gender

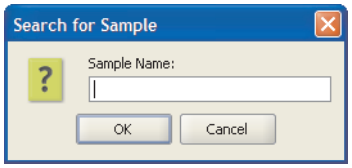
Export Table Data to File

To view data in the Data Table pane:

- To sort data, click a column heading.

  For example, to sort the data by sample name, click **Sample Name**. The assays are sorted by sample name, in alpha-numerical order.
- To search for a sample:
  a. Right-click the **Sample Name** column heading, then click **Search for Sample**.
  b. In the Search for Sample dialog box, enter the sample name, then click **OK**. The software highlights the first row that the sample is found in.

  Note: The Sample Name field is case-sensitive.



- To export the data, click **Export Table Data to File**, enter a file name and type, then click **Save**.
- To print the Data Table pane:
  a. Right-click in the table and select **Print Well Data**.
  b. When prompted, click **OK** in the Print dialog box.

Notes

Statistics tab

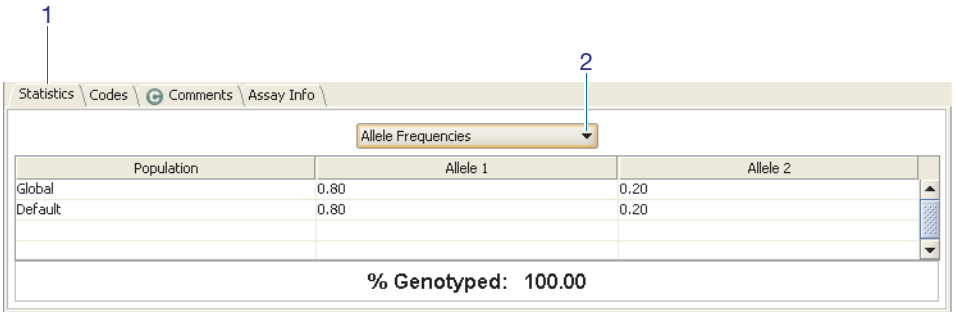
The Statistics tab displays calculated statistics for the selected assay, based on the category you select:

Category	Description
Allele Frequencies	Displays the total number of times (as a percentage) that Allele 1 and Allele 2 occur in the samples for the selected reaction plate or multiplate.
Genotype Distribution	Displays the total number of times (as a percentage) each possible genotype occurs in the samples for the selected reaction plate or multiplate.
Hardy-Weinberg Equilibrium	Displays the Hardy-Weinberg Equilibrium (HWE) for any target populations identified when the study was created ("Create studies" on page 19). The HWE can help you determine: • How to call two cluster plots. • Whether or not an assay is following normal Mendelian rules.
Averages and Standard Deviations	Displays the average signal level and standard deviation for each cluster.
Normalized Averages	Displays the Rn-NTC value for each cluster.
MaxL Metrics	Displays statistics from genotyping called by the software. These values are used in the auto-analysis criteria.

To view statistics in the Statistics tab:

1. Click the **Statistics** tab (default).
2. Select a category from the drop-down menu:
   - **Allele Frequencies**
   - **Genotype Distribution**
   - **Hardy-Weinberg Equilibrium**
   - **Averages and Standard Deviations**
   - **Normalized Averages**
   - **MaxL Metrics**

The display in the Statistics tab changes, depending on the category you select. For example, if you select **Allele Frequencies**, the Statistics tab displays the table below.



Notes

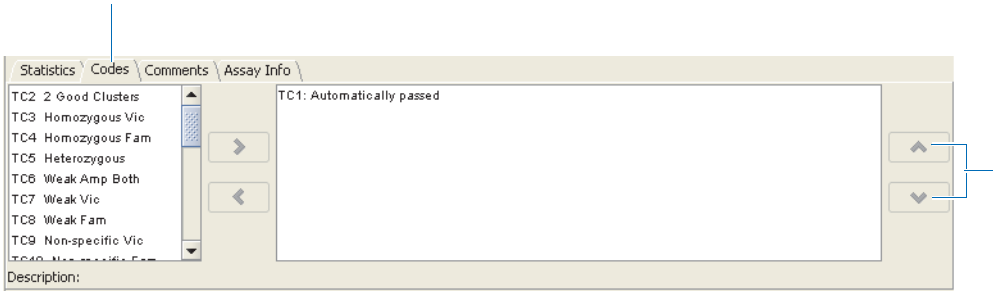
Codes tab

The Codes tab displays the Auto-Codes assigned to the assay, based on the analysis criteria you selected for the study (see "About the automation settings" on page 22). The AutoCaller software automatically assigns Auto-Codes to assays when the SDS file is imported. Users at the Administrator or Scientist User Level can also assign (add or remove) Auto-Codes manually.

Note: If an Auto-Code is manually added or removed by a user, the user's name and the date and time the change was made are recorded in the AutoCaller software database.

- To view codes in the Codes tab, click the **Codes** tab. The Auto-Codes assigned to the assay are displayed in the panel on the right-hand side.

  If there is more than one Auto-Code assigned to the assay, you can move the Auto-Code up or down in the list: Select the Auto-Code, then click the up or down arrow.



- To add or remove an Auto-Code, see "Edit data" on page 73.

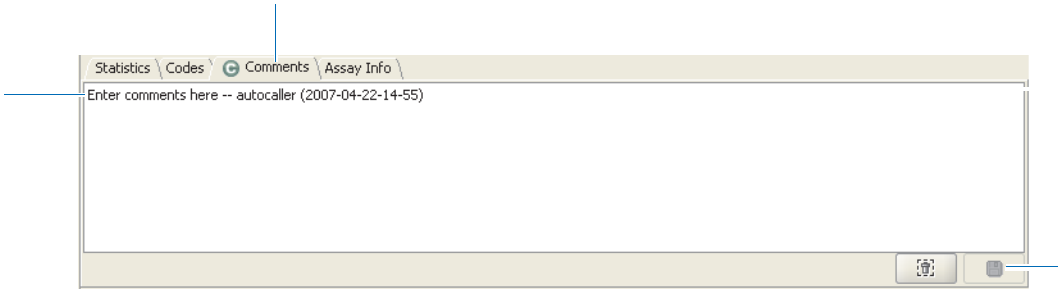
  Note: You must belong to the Administrator or Scientist User Level to add or remove Auto-Codes.

Comments tab

The Comments tab provides space for you to record information about the assay. For example, you may want to record why you manually failed an assay or you may want to leave notes for other users.

- To view or add comments in the Comments tab, click the **Comments** tab. To add comments for the selected assay, enter the information you want to record, then click **Save to database**.

  Note: When you save a comment, the software displays your user name and the date and time you made the comment. Additionally, the software displays in the Comments tab to indicate that comments have been made for the selected assay.



Notes

- To delete all comments for the selected assay, see "Edit data" on page 73.

IMPORTANT! You must belong to the Administrator or Scientist User Level to delete comments.

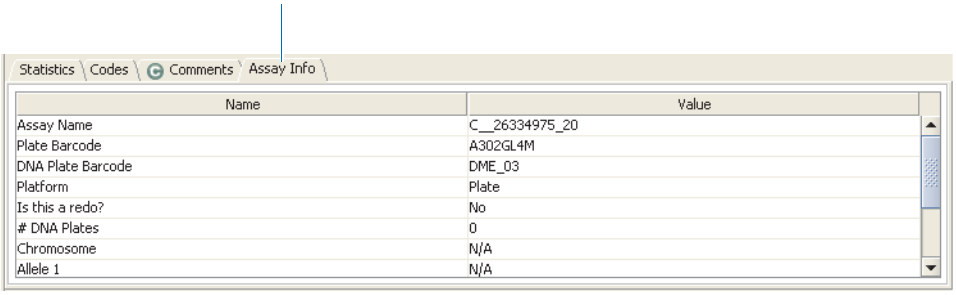
Assay Info tab

The Assay Info tab provides the following information for the selected assay:

Row Name	Description
Assay Name	The assay name or ID number.
Plate Barcode	The name or barcode of the reaction plate in which the assay was run. The name or barcode is entered by the user in the SDS file (page 7).
DNA Plate Barcode	The name or barcode of the source DNA plate (that is, the DNA plate that was used to seed the reaction plate): • *<DNA plate name>* – The software displays the source DNA plate name if the user included the name in the file (page 7) or in the sample set file (page 105). • *MULTI* – The software assigns the name *MULTI* whenever there is more than one plate in the study with the same assay. For more information, see "Select a multiplate" on page 71. • *No DNA Plate* – The software displays *No DNA Plate* for any reaction plates that do not have a name or multiplate assignment.
Platform	The consumable used in the experiment: • **Plate** indicates a 96- or 384-well reaction plate. • **OpenArray** indicates the data was collected on the OpenArray™ NT Imager Genotyping System.
# DNA Plates	For multiplate studies, the number of reaction plates imported (via the SDS file) for the assay.
Chromosome	The chromosome assigned to the assay.
Allele 1	The base assigned to allele 1 of the assay (A, C, G, T, +, or -), if a user selected a base for Actual Allele 1 in the Assay Management window. For more information, see "Edit assay information" on page 41.
Allele 2	The base assigned to allele 2 of the assay (A, C, G, T, +, or -), if a user selected a base for Actual Allele 2 in the Assay Management window. For more information, see "Edit assay information" on page 41.
SNP Comments	Comments entered by a user in the Assay Management window. For more information, see page 41.
Priority Code	The priority code assigned by a user to the assay in the Assay Management window. Priority codes are defined by the user and can be used to facilitate searches (for example, a gene name can be used as a priority code). For more information, see "Edit assay information" on page 41.
Load Date	The date the assay was imported into the AutoCaller software database.
Export Date	The date the assay was exported in *.xml format.

Notes

To view information for the selected assay, click the **Assay Info** tab.



Status menu

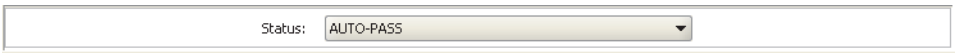
The Status menu displays the analysis status assigned to the selected assay:

Status	Assigned By	Reason
AUTO-PASS	Software	The AutoCaller software assigns AUTO-PASS if the assay passes all of the analysis criteria selected for the study.
AUTO-FAIL		The AutoCaller software assigns AUTO-FAIL if the assay fails one or more of the analysis criteria selected for the study.
HOLD	User	Assign HOLD to an assay when you want to review (or have another user review) the assay before reporting data.
NEW		Assign NEW to an assay newly imported into the AutoCaller software database. Assigning the NEW status is useful when you want to find and review new assays before reporting data.
REPLICATE		Assign REPLICATE to indicate that the assay was run twice on the same sample set.
REDO		Assign REDO to an assay as a way to flag assays for further inspection. You can search for assays with the REDO status in the Search for Assays dialog box.
MANUAL-PASS		Assign MANUAL-PASS to indicate that the assay was passed by a user and not by the software. This is useful if: • The assay failed one or more analysis criteria selected for the study, but you were able to pass the assay after manually calling genotypes. • The assay was imported with the NEW status (that is, no analysis criteria were selected for the study).
MANUAL-FAIL		Assign MANUAL-FAIL to indicate that the assay was failed by a user and not by the software. This status is useful if: • The assay passed all analysis criteria selected for the study, but you want to fail the assay anyway. • You want to confirm an AUTO-FAIL call (that is, you reviewed the AUTO-FAIL call and confirmed the data do not pass). • The assay was imported with the NEW status (that is, no analysis criteria were selected for the study).

Notes

For information on analysis criteria, see "About the automation settings" on page 22.

Use the Status menu (shown below) to view the analysis status assigned to an assay. To manually assign an analysis status, see "Edit data" on page 73.

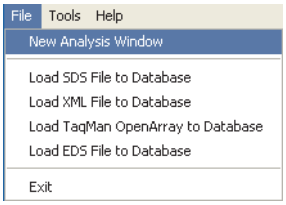


IMPORTANT! You must belong to the Administrator or Scientist User Level to manually assign an analysis status.

Open a second window

Open a second window to compare two runs from the same study or from different studies. There are two ways to open a second window:

- From the main window toolbar, select **File ▸ New Analysis Window**.



- Start the AutoCaller software twice so that you have two instances of the software running. You can either open:
  - The same study in each instance.

    or

  - A different study in each instance.

  This option allows you to easily navigate between studies.

Select a multiplate

The AutoCaller software creates an extra plate (a *multiplate*; abbreviated as *MULTI* in the Assay Selection pane) that contains all the reaction plates within a study for a single assay. Each reaction plate is treated as an individual plate for importing and genotyping (allele calling), but all the plates can be viewed together and edited as a multiplate to save time.

Select a multiplate when you want to compare:

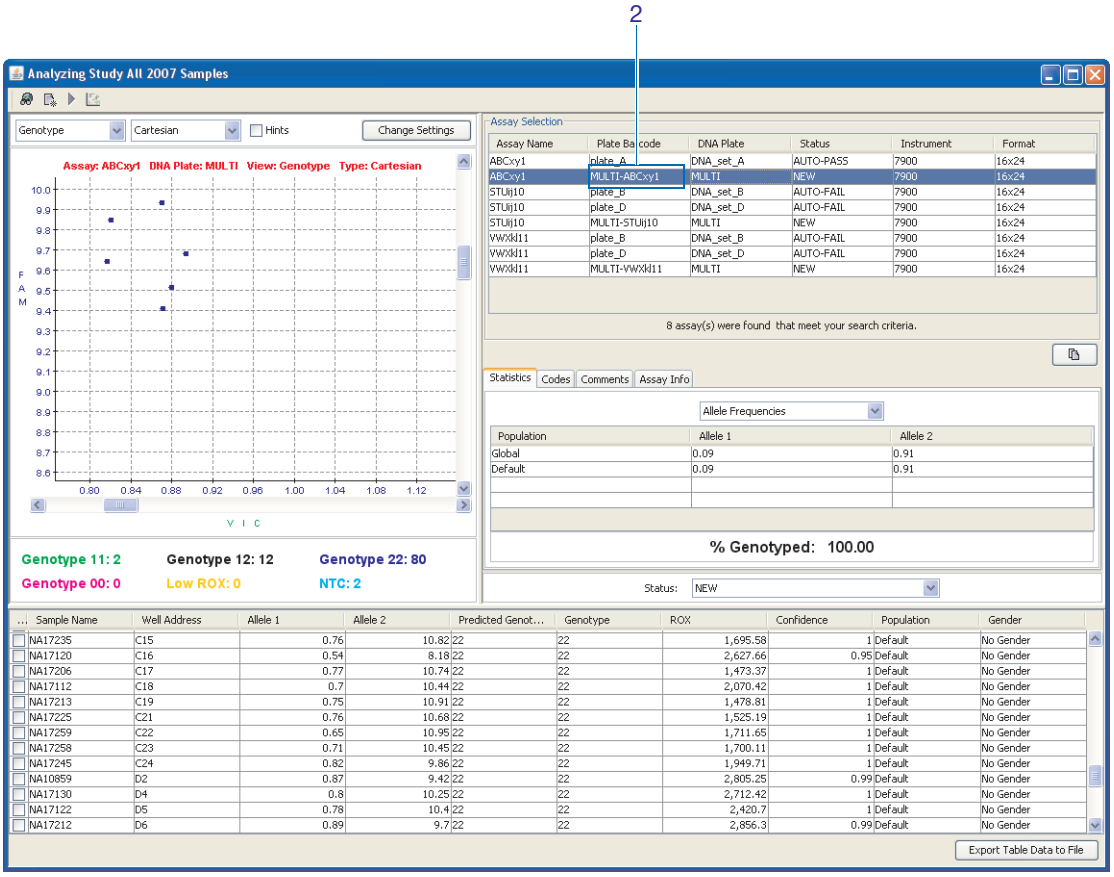
- Duplicate runs (the same assays run on the same samples)
- A multiplate experiment (an experiment with more than 384 samples)

To select a multiplate:

1. In the Assay Selection pane, look for **MULTI** in the Plate Barcode column. (There may be several multiplates; the software creates a multiplate for any assay that was run on more than one reaction plate.)
2. To view the data for the multiplate, select the assay. In the Plot View, the software displays the data for all the plates overlaid in one view.

Notes

3. To return to individual views of each plate, select an individual plate.



Notes

Edit data

You can edit data in the AutoCaller software as follows:

- Edit genotypes (allele calls) (below)
- Edit Auto-Codes (page 75)
- Edit the analysis status (page 76)
- Omit wells from the analysis (page 76)
- Delete comments (page 80)

IMPORTANT! Any edits you make to the data are saved to the AutoCaller software database. Your edits are tracked with your user name and the date and time you made the edits. There is no UNDO feature for edits to the data.

Required user level

To perform the procedures in this section, log in to the AutoCaller software at the Administrator or Scientist User Level.

Edit the genotypes

There are two ways to edit genotypes (allele calls):

- Use the Plot View pane (below)
- Use the Data Table pane (page 74)

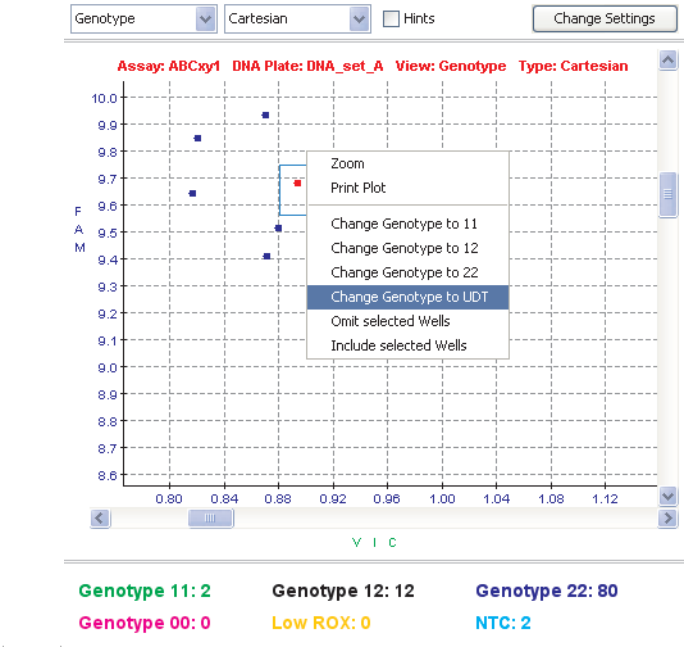
Edit genotypes in the Plot View pane

1. In the Assay Selection pane, select the desired assay. You can select:
   - A single assay to edit the assay for an individual reaction plate.

     or
   - A multiplate to edit the assay across reaction plates. (Look for **MULTI** in the Plate Barcode column. There may be several multiplates; the software creates a multiplate for any assay that was run on more than one reaction plate.)
2. In the Plot View pane, draw a box around the samples (points) to edit.

   Note: When you draw a box around the points in the Plot View pane, the corresponding samples in the Data Table pane are highlighted.
3. Right-click anywhere in the Plot View pane, then select the appropriate option from the pop-up menu:
   - 11 – Homozygous VIC dye
   - 12 – Heterozygous
   - 22 – Homozygous FAM dye
   - UDT – Undetermined genotype

Notes



Edit genotypes in the Data Table pane

1. In the Assay Selection pane, select the desired assay. You can select:
   - A single assay to edit the assay for an individual reaction plate.

     or
   - A multiplate to edit the assay across reaction plates. (Look for **MULTI** in the Plate Barcode column. There may be several multiplates; the software creates a multiplate for any assay that was run on more than one reaction plate.)
2. In the Data Table pane, click the Genotype field for the sample you want to edit.

   Note: You can edit only one sample at a time in the Data Table pane. When you select the sample in the Data Table pane, the corresponding sample (point) in the Plot View pane is highlighted.
3. From the Genotype drop-down menu, select the appropriate option:
   - 11 – Homozygous VIC dye
   - 12 – Heterozygous
   - 22 – Homozygous FAM dye
   - 00 – Undetermined genotype

Sample Name	Row/Column	Allele 1	Allele 2	Predicted Genotype	Genotype	ROX	Confidence	Population	Gender
NA17242	I14	1.45	0.72	11	11	1,793.04	1	Default	No Gender
NA17206	A17	1.45	0.7	11	11	1,794.68	1	Default	No Gender
NA17125	N16	1.45	0.77	11	12	1,614.45	1	Default	No Gender
NA17130	L17	1.46	0.78	11	22	1,821.48	1	Default	No Gender
NA17201	I15	1.47	0.72	11	00	1,738.27	1	Default	No Gender
NA17213	C17	1.47	0.7	11	11	1,540.32	1	Default	No Gender
NA17260	H16	1.47	0.75	11	11	1,818.02	1	Default	No Gender
NA17231	P17	1.47	0.74	11	11	1,630.59	1	Default	No Gender
NA10859	J17	1.47	0.77	11	11	1,669.91	1	Default	No Gender

Notes

Edit the Auto-Codes

In the Assay Selection pane, select the desired assay, then click the **Codes** tab. The Auto-Codes assigned to the assay are displayed in the list on the right-hand side.

To add an Auto-Code to the assay:

1. Select the appropriate Auto-Code from the list on the left side.
2. Click > **Add assay code(s)**.

   You can select one code at a time.

To remove an Auto-Code from the assay:

1. Select the appropriate Auto-Code from the list on the right side.
2. Click < **Remove assay code(s)**.

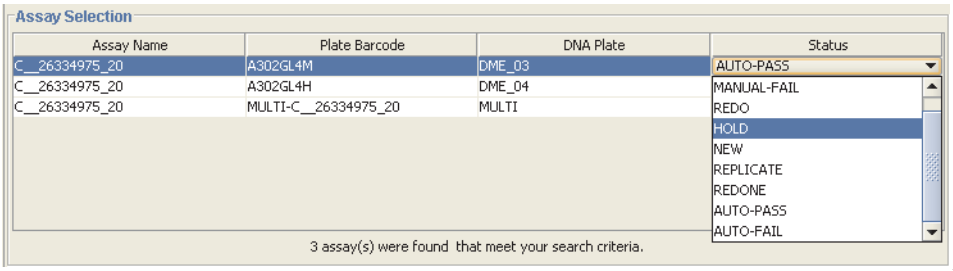
For more information on the Auto-Codes, see "Codes tab" on page 68.

Notes

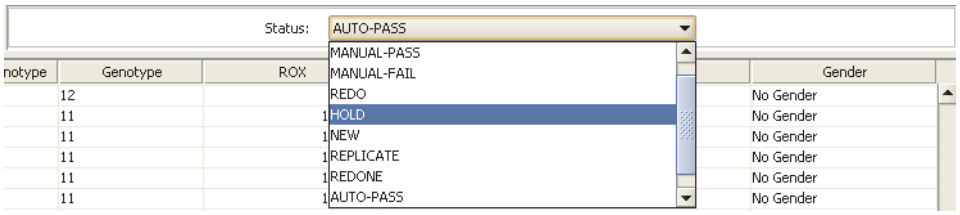
Edit the analysis status

To change the analysis status:

- In the Assay Selection pane, select the assay to edit, click the Status cell for the assay you want to change, then select a status from the drop-down menu.



- In the Status menu, select an analysis status from the drop-down menu.



Note: **AUTO-PASS** and **AUTO-FAIL** are automatically assigned by the AutoCaller software. Users cannot manually set these options.

For more information on the analysis status options, see "Status menu" on page 70.

Omit wells from the analysis

There are three ways to omit wells from analysis:

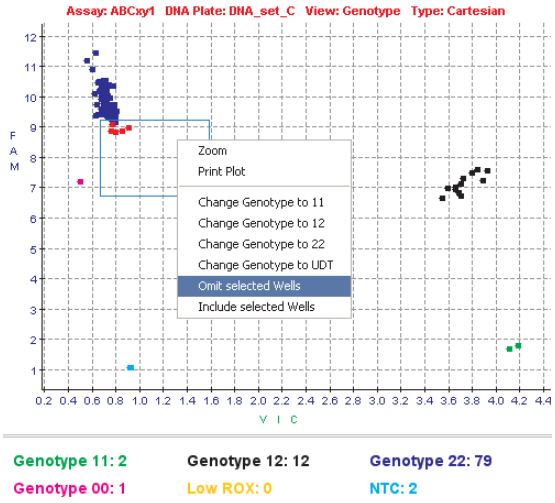
- Use the Plot View pane (below)
- Use the Data Table pane (page 78)
- Use the Data Explorer window, to omit all wells for a given sample (page 79)

Omit wells in the Plot View pane

1. In the Assay Selection pane, select the desired assay. You can select:
   - A single assay to edit the assay for an individual reaction plate.

     or
   - A multiplate to edit the assay across reaction plates. (Look for **MULTI** in the Plate Barcode column. There may be several multiplates; the software creates a multiplate for any assay that was run on more than one reaction plate.)
2. In the Plot View pane, draw a box around the samples (points) to omit. The corresponding samples in the Data Table pane are highlighted.
3. Right-click anywhere in the Plot View pane, then select **Omit Selected Wells** from the pop-up menu:

Notes



The selected well(s) become less intensely shaded and the **...** (Omitted) check box in the Data Table pane is checked. The statistics in the Statistics tab (except for the MaxL Metrics) are immediately recalculated without the values from the omitted wells. The wells remain omitted from any further analysis.

After omitting wells, you can:

- Click (Assign Genotypes) to reassign genotypes and codes, based on the included wells.
- Click (Repeat Auto-Analysis) to reassign only codes based on the included wells.

To include omitted wells:

1. In the Plot View pane, draw a box around the samples (points) to include.
2. Right-click anywhere in the Plot View pane, then select **Include Selected Wells** from the pop-up menu.

   The selected well(s) are reshaded, the **...** (Omitted) check box in the Data Table pane is unchecked, and the statistics in the Statistics tab are immediately recalculated.

Notes

Omit wells in the Data Table pane

1. In the Assay Selection pane, select the desired assay. You can select:
   - A single assay to edit the assay for an individual reaction plate.

     or
   - A multiplate to edit the assay across reaction plates. (Look for **MULTI** in the Plate Barcode column. There may be several multiplates; the software creates a multiplate for any assay that was run on more than one reaction plate.)
2. In the Data Table pane, right-click and select **Omit Selected Wells**.

   The selected well(s) become less intensely shaded and the **...** (Omitted) check box in the Data Table pane is checked. The statistics in the Statistics tab are immediately recalculated without including the values from the omitted wells. The wells remain omitted from any further analysis.

After omitting wells, you can:

- Click  (Assign Genotypes) to reassign genotypes and codes, based on the included wells.
- Click (Repeat Auto-Analysis) to reassign only codes based on the included wells.

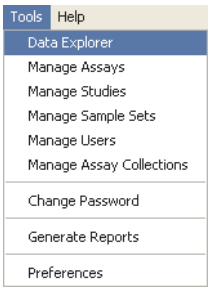
To include omitted wells, in the Data Table pane, right-click, then select **Include Selected Wells** from the pop-up menu.

The selected well(s) are reshaded, the **...** (Omitted) check box in the Data Table pane is unchecked, and the statistics in the Statistics tab are immediately recalculated.

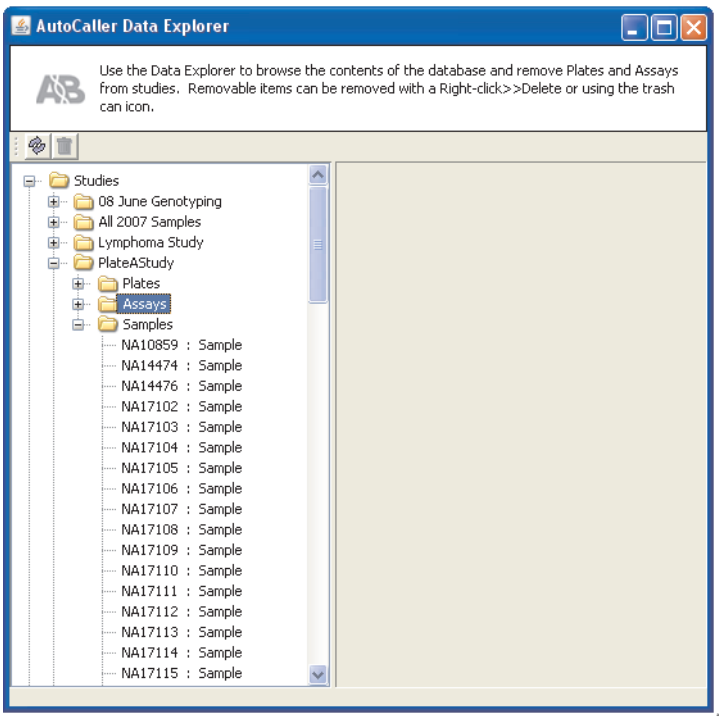
Notes

Omit all wells for a sample

1. Select **Tools ▸ Data Explorer**.

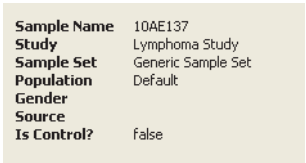


2. In the Data Explorer window, click to open a study, then click Samples to view the samples in the study.



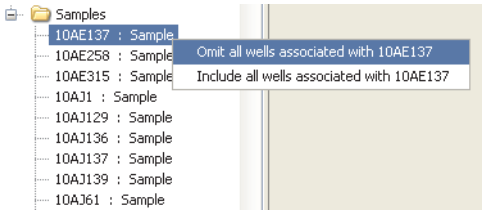
3. Omit the sample:

   a. Click the sample to be omitted. The right side of the Data Explorer window displays information about the sample.

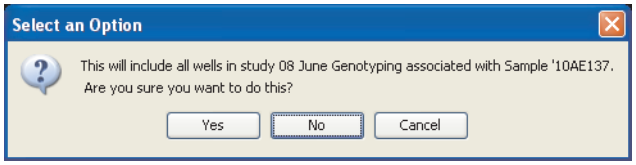


Notes

b. Right-click and select **Omit wells associated with <*sample_name*>**.



c. At the prompt, click **Yes** to omit all wells for the sample.



d. Click **OK** in the message box.

The wells for this sample are omitted from any analysis. In the Analyze window, the selected well(s) are less intensely shaded and the **...** (Omitted) check box in the Data Table pane is checked. The statistics in the Statistics tab are calculated without the values from the omitted wells. The wells remain omitted from any further analysis.

After omitting wells, you can:

- Click (Assign Genotypes) to reassign genotypes and codes, based on the included wells.
- Click (Repeat Auto-Analysis) to reassign only codes based on the included wells.

To include an omitted sample:

1. Click the sample to be included.
2. Right-click and select **Include wells associated with <*sample_name*>**.
3. At the prompt, click **Yes** to include all wells for the sample.
4. Click **OK** in the message box.
5. The wells for this sample are included for any analysis. In the Analyze window, the selected well(s) are reshaded, the **...** (Omitted) check box in the Data Table pane is unchecked, and the statistics in the Statistics tab are calculated with the values from these wells.

When you are finished, click (Close) to close the Data Explorer window.

Delete comments

1. In the Assay Selection pane, select the desired assay.
2. Click the **Comments** tab.

Notes

3. Click **Clear all existing comments**. All comments for the selected assay are cleared.
4. Click **Save to database**.

Note: When you delete the comments, the software displays your user name and the date and time you deleted the comments. Additionally, the software still displays in the Comments tab to indicate that comments had been made for the selected assay.

For more information on comments, see "Comments tab" on page 68.

Notes

Notes

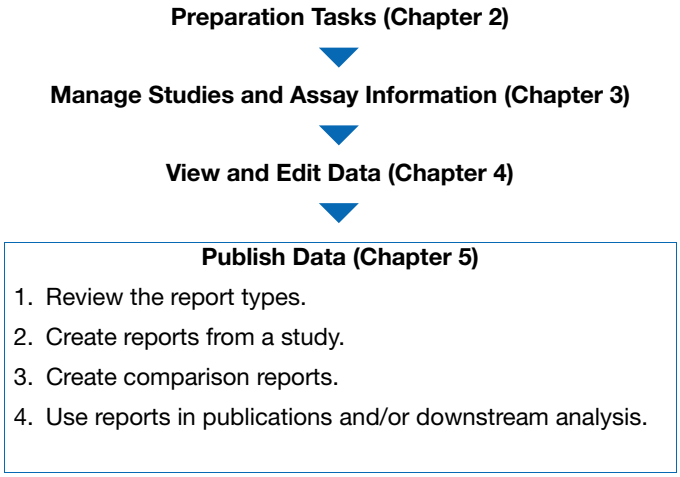
5 Publish Data

This chapter covers:

Chapter overview

This chapter explains how to generate reports from a study in the Applied Biosystems AutoCaller™ Software. This chapter also explains how to generate comparison reports for downstream analysis.

Workflow



Required user levels

To perform the procedures in this chapter, log in to the AutoCaller software at any User Level. For information on User Levels, see "About user levels" on page 3.

Report types

AutoCaller software creates two types of reports:

- Study reports, described on page 85, contain information from a single study,
- Comparison reports, described on page 93, compare information from one study to either another study or a single sample in the study.

Both types of reports are text files.

Notes

Creating a report from a study

The AutoCaller software provides the following report types for data from a study:

- Assay Report (below)
- Assay & Well Report (page 86)
- Assay & Sample Report (page 87)
- Sample Genotype Report (page 88)
- Sample Assay Report (page 88)

These reports are called *study reports* because they contain information from a single study.

To create a study report, see "How to create a study report" on page 89.

Contents of the study reports

Assay Report

For the selected study, the Assay Report contains summary information for each assay, as described below.

	A	B	C	D	E	F	G	H	I	J	K	L	M	
1	Autocaller Version: 3.2.0													
2	assay_name	plate_barcode	dna_plate	plate_location	status	chromosome	allele1	allele2	percent_genotyped	sigma_separation	MAF Default	Minor Allele Default	codes	comments
3	C__26334975_20	A302GL4M	DME_03	1	AUTO-PASS	N/A	N/A	N/A	100	14.88541059	0.244680851	2	TC1, TC4, TC9	null
4	C__26334975_20	A302GL4H	DME_04	1	AUTO-PASS	N/A	N/A	N/A	98.94	11.26291715	0.247311828	2	TC1	Enter comments here --
5														
6	C__26334975_20	MULTI-C__26334975_20	MULTI	0	NEW	N/A	N/A	N/A	99.47	0.245989305	2	N/A	null	
7														
8														

Column	Description
assay_name	The assay name or ID number.
plate_barcode	The name or barcode of the reaction plate in which the assay was run.
dna_plate	The name or barcode of the source DNA plate (that is, the DNA plate that was used to seed the reaction plate): • *<DNA plate name>* – The software displays the source DNA plate name if the user included the name in the file or in the sample set file. • *MULTI* – The software assigns the name *MULTI* whenever there is more than one plate in the study with the same assay. • *No DNA Plate* – The software displays *No DNA Plate* for any reaction plates that do not have a name or a multiplate assignment.
status	The analysis status of the assay)
chromosome	The chromosome assigned to the assay, if a chromosome was selected for this assay.
allele1	The allele being coded with the VIC® dye probe. Allele 1 information is only available if Allele 1 was assigned for this assay.
allele2	The allele being coded with the FAM™ dye probe. Allele 2 information is only available if Allele 2 was assigned for this assay.

Notes

Column	Description
percent_genotyped	The percentage of samples on the reaction plate genotyped for each assay. This is useful for DNA QC statistics; a low percentage might indicate an under-performing assay, a poor-quality sample, or a null allele.
sigma_separation	The measure of cluster separation and cluster tightness.
MAF <*population*>	The total number of times (as a percentage) that a minor allele occurs in the specified population. **Note:** MAF = Minor allele frequency; the minor allele is the least frequent of the two alleles.
Minor Allele <*population*>	The minor allele genotyped for the specified population: 1 or 2. Minor allele information is available only if a minor allele was assigned for this assay. **Note:** If a user selected a base in the Assay Management window, the genotype for each sample is AA, TT, and so on.
codes	The Auto-Code(s) automatically or manually assigned to an assay, as defined by the analysis criterion. For a list of Auto-Codes and the corresponding analysis criteria, see page 22.
comments	Comments entered by a user from the Assay Management window or from the Comments tab o f the Analyze window.

Assay & Well Report

For the selected study, the Assay & Well Report contains summary information for each well of each assay, as described below. The Assay & Well Report is useful for exporting genotypes for downstream analysis.

	A	B	C	D	E	F	G	H	I
1	Autocaller Version: 3.2.0								
2	assay_name	plate_barcode	dna_plate	sample_name	well_number	genotype	confidence	allele_1	allele_2
3	C__26334975_20	A302GL4M	DME_03	NA17214	193	11	0.996325911	1.831281185	0.810485959
4	C__26334975_20	A302GL4M	DME_03	NA10859	194	11	0.999481466	1.760317087	0.908820927
5	C__26334975_20	A302GL4M	DME_03	NA17216	195	11	0.999920814	1.772682667	0.857936502
6	C__26334975_20	A302GL4M	DME_03	NA17261	196	11	0.999978968	1.766975403	0.857633114
7	C__26334975_20	A302GL4M	DME_03	NA17239	200	11	0.990026172	1.883795619	0.909038603
8	C__26334975_20	A302GL4M	DME_03	NA17207	202	11	0.991726793	1.874718428	0.887204111
9	C__26334975_20	A302GL4M	DME_03	NA17226	204	11	0.991134626	1.874757767	0.919510245
10	C__26334975_20	A302GL4M	DME_03	NA17117	205	11	0.996626938	1.832411647	0.832931161
11	C__26334975_20	A302GL4M	DME_03	NA17102	208	11	0.995311366	1.84733665	0.870146632
12	C__26334975_20	A302GL4M	DME_03	NA17242	217	11	0.999038801	1.730233788	0.824412346

Column	Description
assay_name	The assay name or ID number.
plate_barcode	The name or barcode of the reaction plate in which the assay was run.

Notes

Column	Description
dna_plate	The name or barcode of the source DNA plate (that is, the DNA plate that was used to seed the reaction plate): • *<DNA plate name>* – The software displays the source DNA plate name if the user included the name in the file or in the sample set file. • *MULTI* – The software assigns the name *MULTI* whenever there is more than one plate in the study with the same assay. • *No DNA Plate* – The software displays *No DNA Plate* for any reaction plates that do not have a name or a multiplate assignment.
sample_name	The name of the sample on which the assay is being run.
well_number	The reaction plate well in which the assay/sample is located.
genotype	The genotype for each sample: 11, 12, 22, or 00. **Note:** If a user selected a base in the Assay Management window, the genotype for each sample is AA, TT, and so on. For more information, see "Edit assay information" on page 41.
confidence	The confidence value for each sample (point). The confidence value indicates the likelihood that the point belongs to the cluster (the cluster is defined by the software).
allele_1	Rn (normalized reporter) value of the VIC® dye signal.
allele_2	Rn (normalized reporter) value of the FAM™ dye signal.

Assay & Sample Report

For the selected study, the Assay & Sample Report lists the genotype for each sample for each assay, as described below. The Assay & Sample Report is useful for exporting genotypes for downstream analysis; however, it is not recommended if no sample sets are defined. (If no sample sets are defined, use the Assay & Well Report described on page 86 instead.)

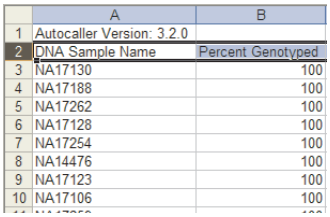
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Autocaller Version: 3.2.0															
2	AssayName	NA10859	NA14474	NA14476	NA17102	NA17103	NA17104	NA17105	NA17106	NA17107	NA17108	NA17109	NA17110	NA17111	NA17112	NA17113
3	C__26334975_20	11	11	12	11	12	12	12	12	12	12	12	12	22	11	12
4	C__26334975_20	11	11	12	11	12	12	12	12	12	12	12	12	22	11	12
5	C__26334975_20	11	11	12	11	12	12	12	12	12	12	12	12	22	11	12
6																

Column	Description
AssayName	The assay name or ID number.
<sample name>	The genotype for each sample: 11, 12, 22, or 00. **Note:** If a user defined the genotypes in the Assay Manager (see "Edit assay information" on page 41), the *<sample name>* is AA, TT, and so on.

Notes

Sample Genotype Report

For the selected study, the Sample Genotype Report shows the percentage of assays that each sample is genotyped for, as described below.



	A	B
1	Autocaller Version: 3.2.0	
2	DNA Sample Name	Percent Genotyped
3	NA17130	100
4	NA17188	100
5	NA17262	100
6	NA17128	100
7	NA17254	100
8	NA14476	100
9	NA17123	100
10	NA17106	100

Column	Description
DNA Sample Name	The name of the sample.
Percent Genotyped	The percentage of assays that each sample is genotyped for. For example, if SampleX was run in 10 assays and was genotyped in 9 of the 10 assays, the Percent Genotyped column displays 90. This is useful for DNA QC statistics; a low percentage might indicate a low-quality sample.

Sample Assay Report

For the selected study, the Sample Assay Report shows the genotype information for a single sample across assays, as described below. For example, if you select SampleX when specifying the export criteria (page 91), the report lists the assays that SampleX was run in and shows how SampleX was genotyped in each of the assays.

	A	B	C
1	Autocaller Version: 3.2.0		
2	Assay Name	Plate Barcode	Genotype
3	C__26334975_20	A302GL4M	12
4	C__26334975_20	A302GL4H	12
5			

Column	Description
Assay Name	The assay name or ID number.
Plate Barcode	The name or barcode of the reaction plate in which the assay was run.
Genotype	The genotype for the selected sample: 11, 12, 22, or 00. **Note:** If a user defined the genotype in the Assay Manager (see "Edit assay information" on page 41), the genotype for the selected sample is AA, TT, and so on.

Notes

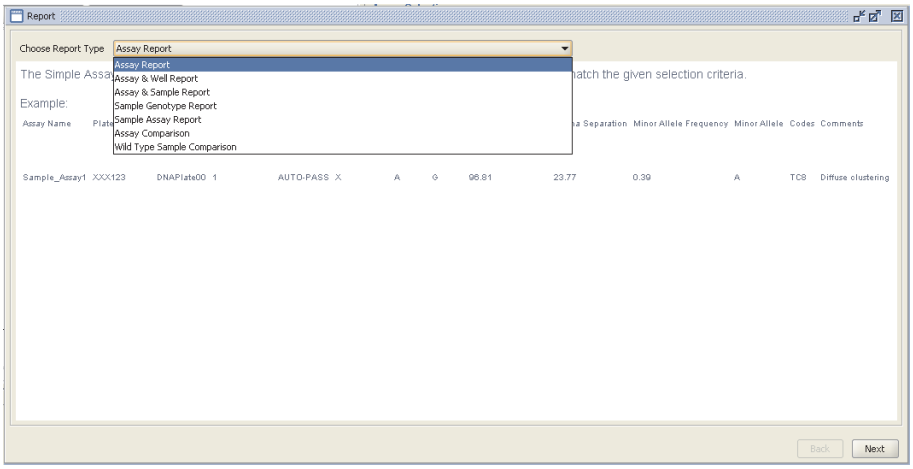
How to create a study report

For each AutoCaller Software report type, the AutoCaller software exports the data from the AutoCaller software database to a text file (*.txt). You can open the text file in a spreadsheet application (such as Microsoft® Excel software) or simple text application (such as Microsoft® Notepad software).

Note: The report provides information only for the assays in the study that meet the export criteria you specify.

1. From the main window toolbar, select **Tools ▸ Generate Reports**.

2. In the Report window:
   a. Select the appropriate report:
      - **Assay Report**
      - **Assay & Well Report**
      - **Assay & Sample Report**
      - **Sample Genotype Report**
      - **Sample Assay Report**
   b. Click **Next**.



3. From the Choose Study drop-down menu, select a study.

Notes

4. (*Optional*) In the Assay Name field, enter the name or ID number of the assay for which you want to export data.

   You can use an asterisk (*) as a wild card. For example, if you enter ***245***, the software exports data for all assays with names that include a 245 sequence: C__23**245**6789_20, C__33**245**6789_20, and so on.

   If you do not specify the assay name or ID number, the software exports data for all assays in the study.

5. *(Optional)* In the Assay Collection dropdown menu, select:
   - The assay collection in which you want to export data.
   - **Define a new collection** to define a new assay collection for use as a search criterion. See page 48 for instructions.

6. (*Optional*) In the Plate Barcode field, enter the barcode for the reaction plate.

   Note: Entering a barcode is useful when you want to export data for all assays run on a single reaction plate.

Notes

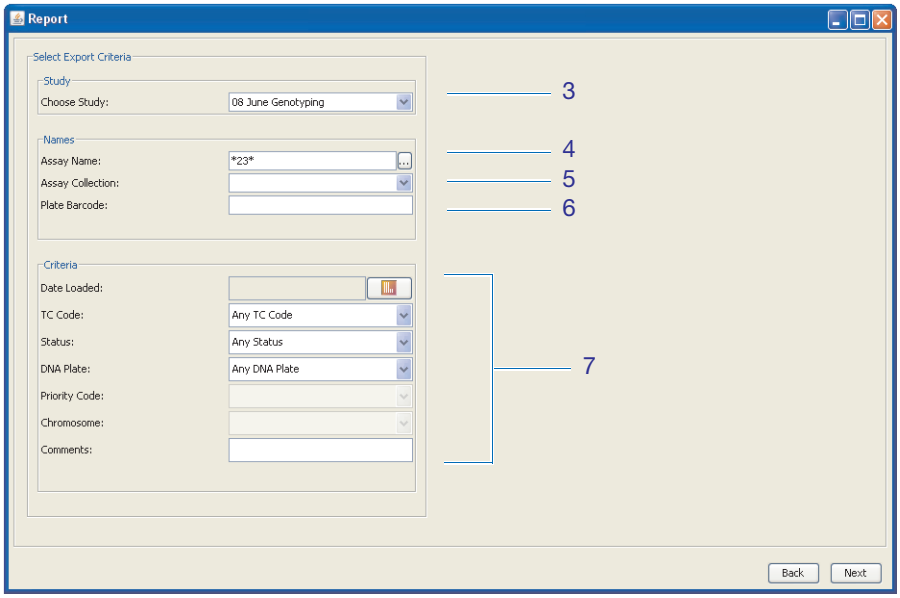
7. (*Optional*) In the Criteria pane, specify any of the following criteria to narrow the set of assays exported:

Field	To specify this criterion...
Date Loaded	Click  **Choose Date Assays Were Loaded**, then select the date the assay was imported into the AutoCaller software database.
TC Code	Select the Auto-Code from the drop-down menu.
Status	Select the analysis status from the drop-down menu.
DNA Plate	Select the source DNA plate name from the drop-down menu: • ***<DNA plate name>*** (user-defined) • **MULTI** • **No DNA Plate** **Note:** Applied Biosystems recommends that you select **MULTI**. If you select **MULTI**, the software exports all data for the selected assay(s) once for the multiplate. If you select **No DNA Plate**, the software exports all data for the selected assay(s) twice: once for the multiplate and once for the individual plates.
Priority Code	Select a priority code from the drop-down menu.
Chromosome	Select a chromosome from the drop-down menu.
Comments	Click the **Comments** field, then enter any comments that appear in the Assay Management window or the Comments tab of the Analyze window. Enter the comments exactly as they appear in the Assay Management or Analyze window, or enter an asterisk (*) as a wild card.

For more information on each criterion, see "About the search criteria" on page 57.

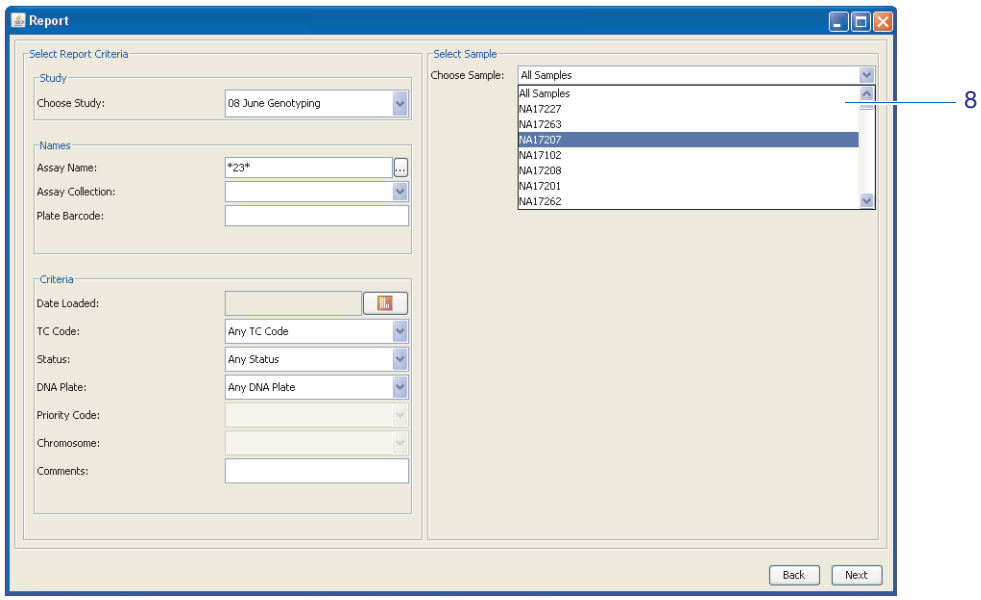
Note: If you do not specify any criteria, the software exports data for all assays with the name or ID number you entered in step 4. If you did not enter an assay name or ID number, the software exports data for all assays in the study.

Notes



8. (*For the Sample Assay Report only*) Select a sample from the Choose Sample drop-down menu.

Note: If you select **All Samples**, the software generates a separate report for *each* sample, with information for each assay selected in the search.



9. Click **Next**.

10. Click **Browse**, then navigate to and select a destination folder.

Notes

11. Click **Report**. The AutoCaller software:
    - Displays the results in the Report window.
    - Exports the data as a text (*.txt) file and saves the file to the selected destination folder.
12. Click **Close** to close the Report window.
13. Open the destination folder to confirm that the export was successful. The *.txt file name includes the report type, the date and time the report was exported, and the study name. For Sample Assay Reports, the file name also includes the sample name.
14. Open the *.txt file in a spreadsheet or simple text application.

Creating a comparison report

The AutoCaller software can generate two types of Comparison Reports:

- Assay Comparison Report (below)
- Wild Type Sample Comparison Report (page 95)

These reports are called *comparison reports* because they compare information from one study to either another study or a single sample in the study

To create a comparison report, see "How to create a comparison report" on page 96.

Contents of the comparison reports

Assay Comparison Report

The Assay Comparison Report compares genotypes between identical samples or assays in two different studies. The Assay Comparison Report is useful when you want to:

- Compare the same assay run with the same samples, but with different run conditions (for example, different master mix, different amounts of DNA, and so on).
- Run control samples on every reaction plate, then compare the results to a validation study.

The Assay Comparison Report is exported as two text (*.txt) files:

- A comparison *.txt file that contains the results of the comparison.
- A mismatches *.txt file that contains a summary of discrepancies.

Note: The reports provide information only for the assays in the studies that meet the report criteria you specify (see "Generate an Assay Comparison Report" on page 96).

Notes

Comparisons *.txt file

	A	B	C	D	E	F	G	H	I	J	K
1	Oligo Name	S1 DNA Plate	S2 DNA Plate	# Wells	% Matched	% Mismatched	% UDTs	S2_00/S1_00	S2_00/S1_11	S2_00/S1_12	S2_00/S1_22
2	C__29712728_10	MULTI	MULTI	94	100	0	0	0	0	0	0
3	C__31366037_10	MULTI	MULTI	94	100	0	0	0	0	0	0
4	C__31366086_10	MULTI	MULTI	94	100	0	0	0	0	0	0
5	C__31366087_10	MULTI	MULTI	94	100	0	0	0	0	0	0
6	C__31366214_30	MULTI	MULTI	94	100	0	1.063829787	1	0	0	0
7	C__33171049_10	MULTI	MULTI	94	0	100	0	0	0	0	0
8	C__33291755_10	MULTI	MULTI	94	100	0	0	0	0	0	0
9	C__34275590_10	MULTI	MULTI	94	100	0	1.063829787	0	0	0	0
10											

Column	Description
Oligo Name	The assay name or ID number.
S1 DNA Plate S2 DNA Plate	For Study 1 (S1 DNA Plate) and Study 2 (S2 DNA Plate), the name or barcode of the source DNA plate (that is, the DNA plate that was used to seed the reaction plate): • *<DNA plate name>* – The software displays the source DNA plate name if the user included the name in the file or in the sample set file. • *MULTI* – The software assigns the name *MULTI* whenever there is more than one plate in the study with the same assay. • *No DNA Plate* – The software displays *No DNA Plate* for any reaction plates that do not have a name or a multiplate assignment.
# Wells	The number of wells in the reaction plate that contain matching samples/assays.
% Matched	The percentage of samples in Study 1 with genotypes that match the sample genotypes in Study 2.
% Mismatched	The percentage of samples in Study 1 with genotypes that do not match the sample genotypes in Study 2.
% UDT	The percentage of samples in Study 1 with undetermined genotypes.
Remaining columns	The remaining columns list every possible combination of genotyping comparisons. The legend is as follows: • S1 = Study 1 • S2 = Study 2 • 00 – Undetermined genotype • 11 – Homozygous VIC dye • 12 – Heterozygous • 22 – Homozygous FAM dye For each assay, the software lists the number of samples that falls into each comparison category. For example, column *S2_00/S1_11* lists the number of samples for each assay that have undetermined genotypes in Study 2 and homozygous VIC dye genotypes in Study 1. In the comparison *.txt file illustrated above, assay C_29712728_10 contains 0 samples that meet this criteria.

Notes

Mismatches *.txt file

	A	B	C	D	E	F	G	H
1	Assay Name	DNA Name	DNA Plate	S1 Well Num	S1 Genotype	Prod DNA Plate	Prod Well Num	Prod Genotype
2	C__33171049_10	NA17214	null	289	11	null	289	22
3	C__33171049_10	NA10859	null	290	11	null	290	22
4	C__33171049_10	NA17216	null	291	11	null	291	22
5	C__33171049_10	NA17261	null	292	11	null	292	22
6	C__33171049_10	NA17227	null	293	11	null	293	22
7	C__33171049_10	NA17263	null	294	11	null	294	22
8	C__33171049_10	NA17137	null	295	11	null	295	22
9	C__33171049_10	NA17239	null	296	11	null	296	22

Column	Description
Assay Name	The assay name or ID number.
DNA Name	The sample name or ID number.
DNA Plate Prod DNA Plate	For Study 1 (DNA Plate) and Study 2 (Prod DNA Plate), the name or barcode of the source DNA plate (that is, the DNA plate that was used to seed the reaction plate): • *<DNA plate name>* – The software displays the source DNA plate name if the user included the name in the file or in the sample set file. • *MULTI* – The software assigns the name *MULTI* whenever there is more than one plate in the study with the same assay. • *null* – The software displays *null* for any reaction plates that do not have a name or multiplate assigned.
S1 Well Num Prod Well Num	For Study 1 (S1 Well Num) and Study 2 (Prod Well Num), the reaction plate well numbers for the samples with mismatched genotypes.
S1 Genotype Prod Genotype	For Study 1 (S1 Genotype) and Study 2 (Prod Genotype), the genotypes of the mismatched samples.

Wild Type Sample Comparison Report

The Wild Type Sample Comparison Report is useful for backcrossing experiments. For the selected study, the Wild Type Sample Comparison Report:

- Compares the selected control sample (the wild type) to the remaining samples in all assays. The remaining samples are identified as being like the wild type or different from the wild type.
- Sums the number of times each sample is like the wild type, then calculates the percentage across all assays in the study.

For example, if you select SampleX as the control sample, the software compares SampleX to the remaining samples in each assay. If SampleY is run in three assays and is like SampleX in all three assays, the total number of times SampleY is like SampleX is 3 and the percentage is 100.

Note: The report provides information only for the assays in the study that meet the report criteria you specify (see "Generate a Wild Type Sample Comparison Report" on page 99).

Notes

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Autocaller Version: 3.2.0													
2	AssayName	NA17108	NA10859	NA14474	NA14476	NA17102	NA17103	NA17104	NA17105	NA17106	NA17107	NA17108	NA17109	NA17110
3	C__26334975_20	1	0	0	1	0	1	1	1	1	1	1	1	0
4	C__26334975_20	1	0	0	1	0	1	1	1	1	1	1	1	0
5	C__26334975_20	1	0	0	1	0	1	1	1	1	1	1	1	0
6	Totals	3	0	0	3	0	3	3	3	3	3	3	3	0
7	Percentage	100	0	0	100	0	100	100	100	100	100	100	100	0
8														

Column/Tow	Description
AssayName	The assay name or ID number.
<sample ID>	Wild type score for each sample: 1 = The sample is like the wild type. 0 = The sample is different from the wild type. N/A = No genotype called for the sample.
Totals	The total number of assays where the sample genotype is identical to the wild type.
Percentage	The number of times (as a percentage) the sample is like the wild type.

How to create a comparison report

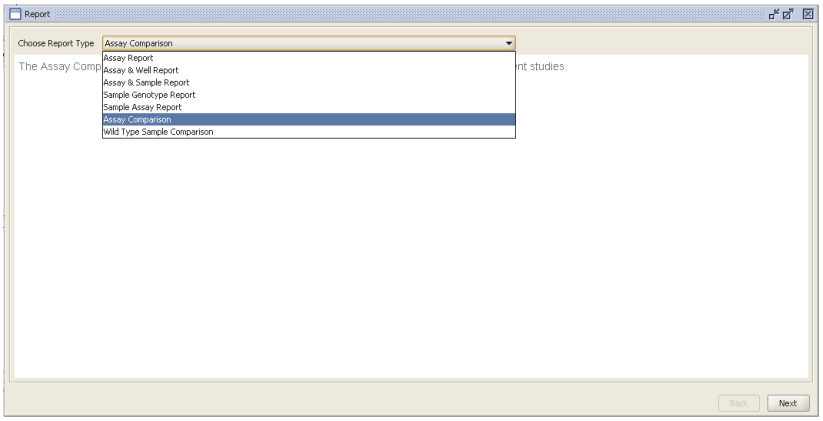
The software exports data from the Comparison Reports as text (*.txt) files. You can open the exported data in a spreadsheet application (such as Microsoft® Excel software) or simple text application (such as Microsoft® Notepad software).

Generate an Assay Comparison Report

1. In the main window toolbar, select **Tools ▸ Generate Reports**.

Notes

2. In the Report window, select **Assay Comparison**, then click **Next**.



3. Select the report criteria for Study 1:

   a. From the Choose Study drop-down menu, select Study 1. Study 1 is the first of the two studies you want to compare.

   Note: For Study 1, Applied Biosystems recommends that you select the study with fewer assays and compare it to the bigger study with the greater number of assays). The AutoCaller software lists all assays in Study 1 and compares them to any identical assays in Study 2. For example, if Study 1 contains 10 assays that meet the report criteria and Study 2 contains 100, the software performs a comparison for those 10 assays in Study 1. If Study 1 contains 100 assays that meet the report criteria and Study 2 contains 10, the software performs a comparison for all 100 assays in Study 1.

   b. (*Optional*) In the Assay Name field, enter the name or ID number of the assay for which you want to export data.

   You can use an asterisk (*) as a wild card. For example, if you enter ***245***, the software exports data for all assays with names that include a 245 sequence: C__23**245**6789_20, C__33**245**6789_20, and so on.

   If you do not specify the assay name or ID number, the software exports data for all assays in the study.

   c. *(Optional)* In the Assay Collection drop-down menu, select the assay collection for which you want to export data.

   d. (*Optional*) In the Plate Barcode field, enter the barcode for the reaction plate.

   Note: Entering a barcode is useful when you want to export data for all assays run on a single reaction plate.

Notes

e. (*Optional*) In the Criteria pane, specify any of the following criteria to narrow the set of assays exported:

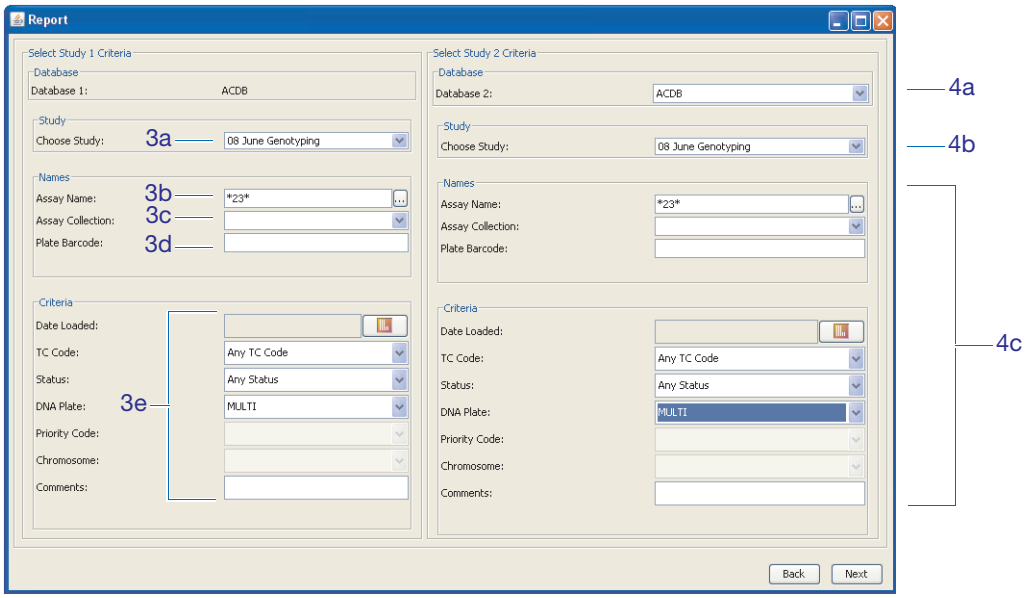
Field	To specify this criterion...
Date Loaded	Click **Choose Date Assays Were Loaded**, then select the date the assay was imported into the AutoCaller software database.
TC Code	Select the Auto-Code from the drop-down menu.
Status	Select the analysis status from the drop-down menu.
DNA Plate	Select the source DNA plate name from the drop-down menu: • ***<DNA plate name>*** (user-defined) • **MULTI** • **No DNA Plate** **Note:** Applied Biosystems recommends that you select **MULTI**. If you select **MULTI**, the software exports all data for the selected assay(s) once for the multiplate. If you select **No DNA Plate**, the software exports all data for the selected assay(s) twice: once for the multiplate and once for the individual plates.
Priority Code	Select a priority code from the drop-down menu.
Chromosome	Select a chromosome from the drop-down menu.
Comments	Click the **Comments** field, then enter any comments that appear in the Assay Management window or the Comments tab of the Analyze window. Enter the comments exactly as they appear in the Assay Management or Analyze window, or enter an asterisk (*) as a wild card.

For more information on each criterion, see "About the search criteria" on page 57.

Note: If you do not specify any criteria, the software exports data for all assays with the name or ID number you entered in step 3b. If you did not enter an assay name or ID number, the software exports data for all assays in the study.

4. Select the report criteria for Study 2:
   a. From the Database 2 drop-down menu, select **ACDB**.
   b. From the Choose Study drop-down menu, select Study 2. Study 2 is the study you want to compare Study 1 to.
   c. Repeat steps 3b through 3e for Study 2.

Notes



5. Click **Next**.
6. Click **Browse**, then navigate to and select a destination folder.
7. Click **Report**. The AutoCaller software:
   - Displays the results in the Report window.
   - Exports the data as two *.txt files.
8. Click **Close** to close the Report window.
9. Open the destination folder to confirm that the export was successful. The AutoCaller software produces two files:
   - The comparison *.txt file contains the results of the comparison.
   - The mismatches *.txt file contains a summary of discrepancies.

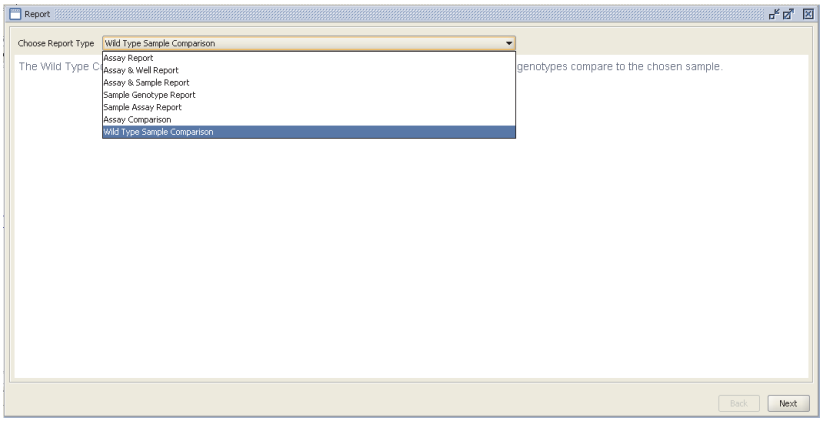
   The *.txt file names include the report type (*comparison* or *mismatches*), the date and time, the name of Study 1, and the name of Study 2.
10. Open the *.txt files in a spreadsheet or simple text application.

Generate a Wild Type Sample Comparison Report

1. From the main window toolbar, select **Tools** ▸ **Generate Reports**.

Notes

2. In the Report window, select **Wild Type Sample Comparison**, then click **Next**.



3. From the Choose Study drop-down menu, select a study.

4. (*Optional*) In the Assay Name field, enter the name or ID number of the assay for which you want to export data.

   You can use an asterisk (*) as a wild card. For example, if you enter ***245***, the software exports data for all assays with names that include a 245 sequence: C__23**245**6789_20, C__332456789_20, and so on.

   If you do not specify the assay name or ID number, the software exports data for all assays in the study.

5. *(Optional)* In the Assay Collection drop-down menu, select:
   - The assay collection in which you want to export data.
   - **Define a new collection** to define a new assay collection for use as a search criterion. See page 48 for instructions.

6. (*Optional*) In the Plate Barcode field, enter the barcode for the reaction plate.

Note: Entering a barcode is useful when you want to export data for all assays run on a single reaction plate.

Notes

7. (*Optional*) In the Criteria pane, specify any of the following criteria to narrow the set of assays exported:

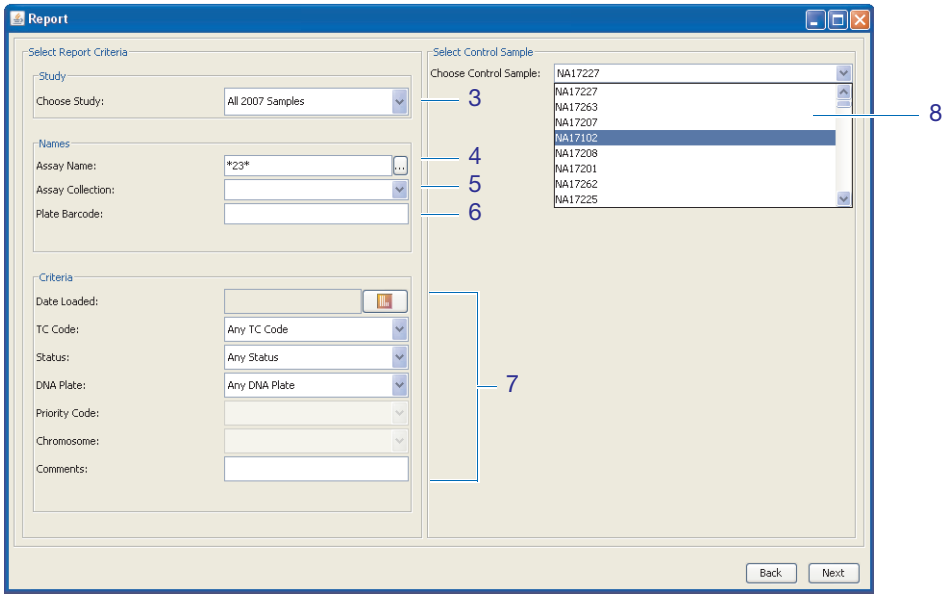
Field	To specify this criterion...
Date Loaded	Click  **Choose Date Assays Were Loaded**, then select the date the assay was imported into the AutoCaller software database.
TC Code	Select the Auto-Code from the drop-down menu.
Status	Select the analysis status from the drop-down menu.
DNA Plate	Select the source DNA plate name from the drop-down menu: • ***<DNA plate name>*** (user-defined) • **MULTI** • **No DNA Plate** **Note:** Applied Biosystems recommends that you select **MULTI**. If you select **MULTI**, the software exports all data for the selected assay(s) once for the multiplate. If you select **No DNA Plate**, the software exports all data for the selected assay(s) twice: once for the multiplate and once for the individual plates.
Priority Code	Select a priority code from the drop-down menu.
Chromosome	Select a chromosome from the drop-down menu.
Comments	Click the **Comments** field, then enter any comments that appear in the Assay Management window or the Comments tab of the Analyze window. Enter the comments exactly as they appear in the Assay Management or Analyze window, or enter an asterisk (*) as a wild card.

For more information on each criterion, see "About the search criteria" on page 57.

Note: If you do not specify any criteria, the software returns all assays with the name or ID number you entered in step 4. If you did not enter an assay name or ID number, the software returns all assays in the study.

Notes

8. From the Choose Control Sample drop-down menu, select a control sample (wild type) that you want to compare the study to.



9. Click **Next**.

10. Click **Browse**, then navigate to and select a destination folder.

11. Click **Report**. The AutoCaller software:
    - Displays the results in the Report window.
    - Exports the data as a text (*.txt) file and saves the file to the selected destination folder.

12. Click **Close** to close the Report window.

13. Open the destination folder to confirm that the export was successful. The *.txt file name includes the report type (*wild_type_comparison*), the date and time the report was exported, and the study name.

14. Open the *.txt file in a spreadsheet or simple text application.

Notes

A

Manage Sample Set Files

This appendix covers:

Appendix overview

This appendix explains how to create sample set files (*.txt format), import the files into the Applied Biosystems AutoCaller™ Software database, and manage the sample sets in the AutoCaller software. This appendix also explains how to create a study using sample sets.

Workflow

Manage Sample Set Files (Appendix A)

1. Create a sample set file for multiplate studies
2. Import the sample set file into the AutoCaller software database.
3. Manage the sample set file in the AutoCaller software.
4. Create a study using the sample set file.

About sample set files

Sample set files are text (*.txt) files that contain information about each sample in a reaction plate. You create sample set files in a text application, then import the files into the AutoCaller software database. When you import the files, the AutoCaller software updates the sample information for any SDS file that included sample information for the reaction plate. Sample set files allow you to:

- Track individual reactions plates by sample, which can help you identify under-performing plates.
- Calculate statistics and alter displays based on population and gender information.

Required user levels

To perform the procedures in this appendix, you must log in to the AutoCaller software at the Administrator or Scientist User Level. For information on User Levels, see "About user levels" on page 3.

Notes

Create a sample set file

You can create sample set files for a multiplate study.

IMPORTANT! To use a sample set in an AutoCaller software study, create and import the sample set file into the AutoCaller software *before* you create the study (page 112).

Create a sample set file for a multiplate study

1. Open a spreadsheet program (such as Microsoft® Excel software).
2. On line one, enter a name for the text file.
3. On line two, enter names for each source DNA plate in the study (for example, **DME_03**, **DME_04**, and so on).

   IMPORTANT! If you are performing duplicate runs of the same data, be sure to give the duplicates different plate names. If you use the same plate name for each duplicate, the AutoCaller software does not import the duplicates (the software assumes the data has already been imported).

4. On the following lines, enter information for each sample. You can list the samples in any order, but you must follow these parameters:
   - Include all samples and all NTCs from all the reaction plates in the study.
   - Enter one sample per line.
   - Enter the sample name.
   - (*Optional*) Enter the population, gender, and source information for each sample.

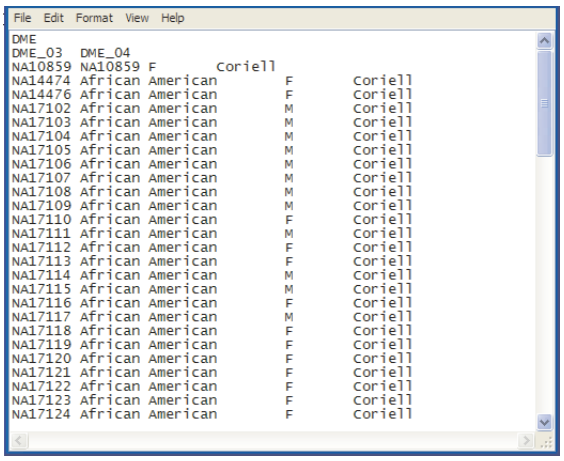
   Note: You do not need to include population, gender, or source information in the *.txt file. If you do not include population information, the AutoCaller software calculates statistics (allele frequency, genotype frequency, and so on) for all samples.

2
3
4

	A	B	C	D
1	DME			
2	DME_03	DME_04		
3	NA10859	NA10859	F	Coriell
4	NA14474	African American	F	Coriell
5	NA14476	African American	F	Coriell
6	NA17102	African American	M	Coriell
7	NA17103	African American	M	Coriell
8	NA17104	African American	M	Coriell
9	NA17105	African American	M	Coriell
10	NA17106	African American	M	Coriell
11	NA17107	African American	M	Coriell
12	NA17108	African American	M	Coriell
13	NA17109	African American	M	Coriell
14	NA17110	African American	F	Coriell
15	NA17111	African American	M	Coriell
16	NA17112	African American	F	Coriell
17	NA17113	African American	F	Coriell
18	NA17114	African American	M	Coriell
19	NA17115	African American	M	Coriell
20	NA17116	African American	F	Coriell
21	NA17117	African American	M	Coriell
22	NA17118	African American	F	Coriell
23	NA17119	African American	F	Coriell
24	NA17120	African American	F	Coriell

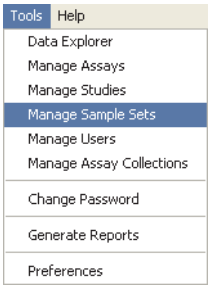
Notes

5. Save the spreadsheet as a text (*.txt) file.

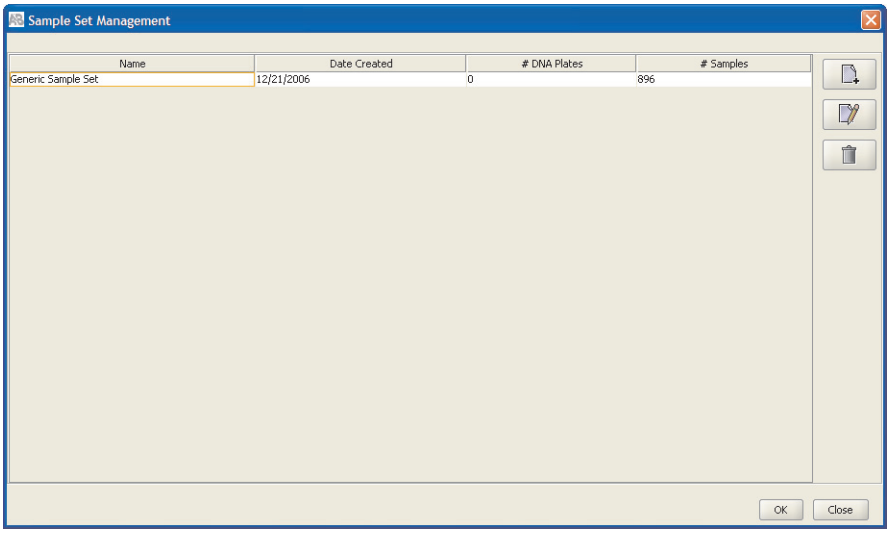


Import a sample set file

1. From the main window toolbar, select **Tools ▸ Manage Sample Sets**.

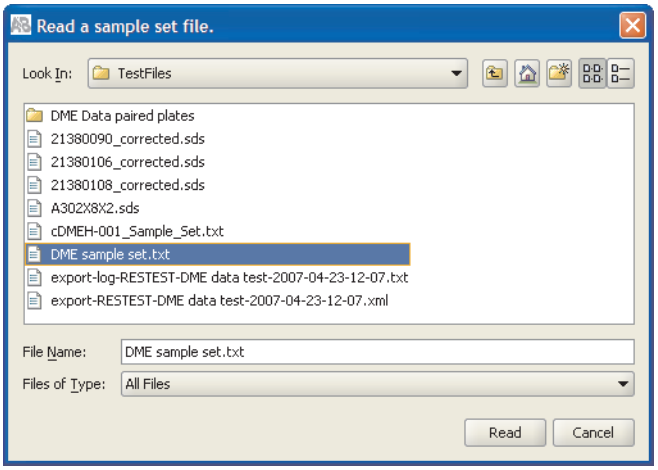


2. In the Sample Set Management window, click  **Add a new sample set to the database**.



Notes

3. In the Read a sample set file dialog box, browse to and select a sample set file (*.txt), then click **Read**.



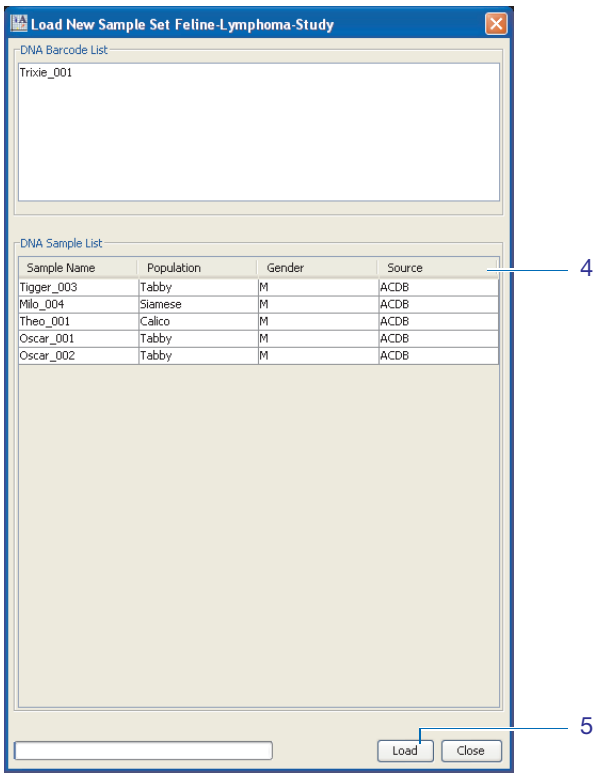
4. In the Load New Sample Set dialog box, review the sample set information for accuracy. If needed, you can edit the sample set information as follows:

Column	Description	To edit...
Sample Name	The name of the sample.	N/A. You cannot edit the sample names.
Population	The population from which the sample is from.	Double-click the Population field for a sample, then enter the new population name.
Gender	The gender from which the sample is from.	Click the Gender field for a sample, then select a gender from the drop-down menu.
Source	The source of the DNA sample (that is, where the DNA came from).	Double-click the Source field for a sample, then enter a new source.

Note: Any edits you make are imported into the AutoCaller software database. However, they are not saved to the original sample set file (*.txt file).

Notes

5. Click **Load** to import the sample set file into the AutoCaller software database.



6. When the sample set file is imported, *Load completed successfully* appears in the Load New Sample Set dialog box. Click **Close** to close the Load New Sample Set dialog box.

7. The new sample set appears in the Sample Set Management window. Click **Close** to close the Sample Set Management window.

Notes

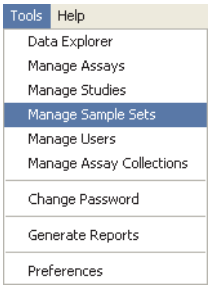
Manage sample sets

The Sample Set Management window displays a list of all sample sets in the AutoCaller software database. You can use the Sample Set Management window to:

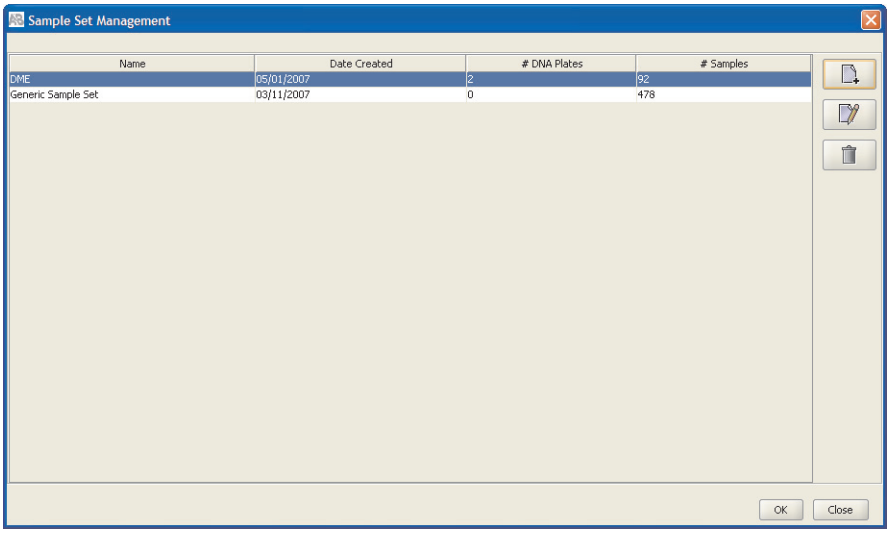
- Add a sample set (as described in "Import a sample set file" on page 106).
- View or edit a sample set (below).
- Delete a sample set from the database (page 111).

View or edit a sample set

1. From the main window toolbar, select **Tools** ▸ **Manage Sample Sets**.



2. In the Sample Set Management window, select the sample set you want to edit, then click  **Edit an existing sample set**.



3. In the Edit Existing Sample Set dialog box, check the sample set information for accuracy.

4. To add a new reaction plate to the sample set:

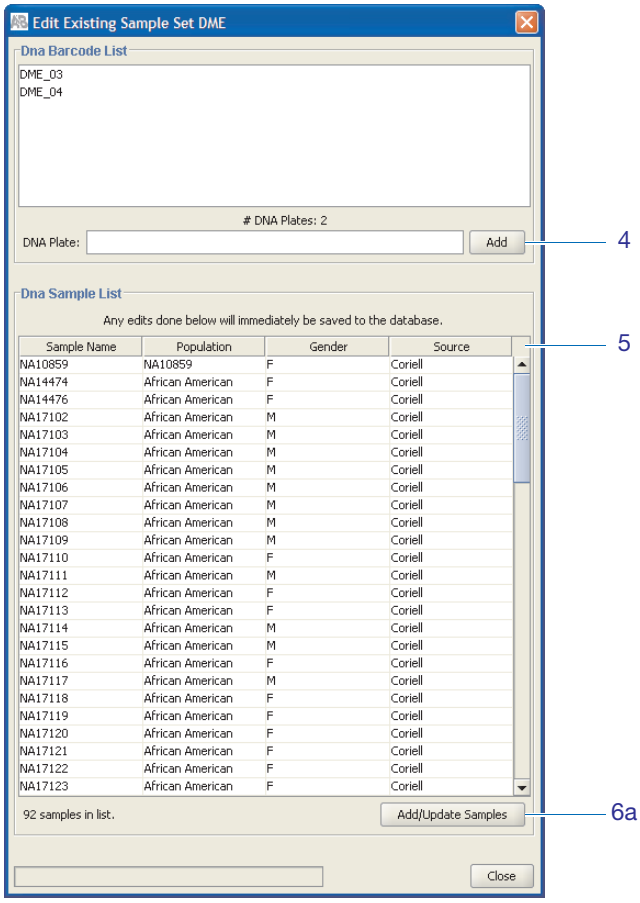
   a. In the DNA Plate field, enter the DNA plate name (user-defined).

   b. Click **Add**.

Notes

5. To edit the sample set information:

Column	Description	To edit...
Sample Name	The name of the sample.	N/A. You cannot edit the sample names.
Population	The population from which the sample is from.	Double-click the Population field for a sample, then enter the new population name.
Gender	The gender from which the sample is from.	Click the Gender field for a sample, then select a gender from the drop-down menu.
Source	The source of the DNA sample (that is, where the DNA came from).	Double-click the Source field for a sample, then enter a new source.



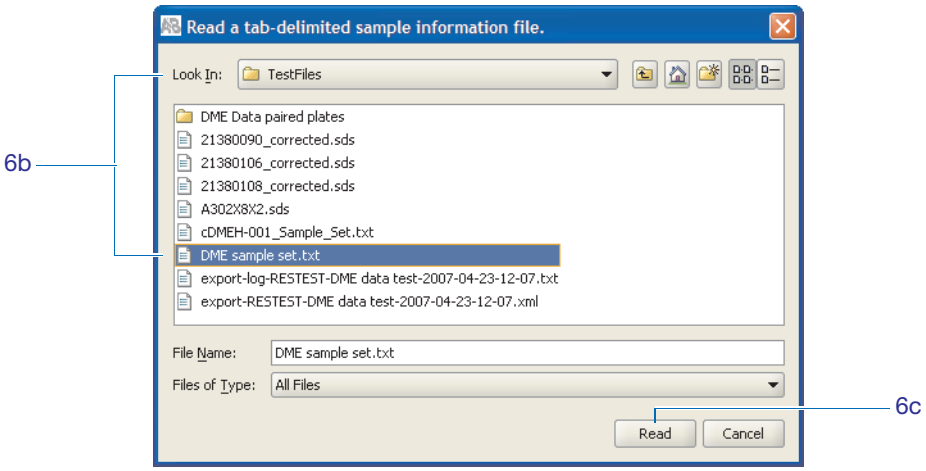
6. To add a sample set file or to update the AutoCaller software database after you have made changes to the original sample set file (*.txt):

   a. Click **Add/Update Samples** to open the Read a tab-delimited sample information file dialog box.

   b. Browse to and select a sample set file (*.txt).

   c. Click **Read** to add or update the sample set file information.

Notes

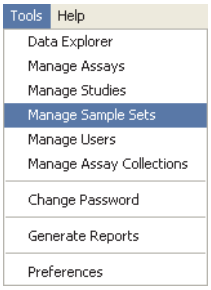


7. Click **Close** to save your changes and close the Edit Existing Sample Set dialog box.
8. Click **Close** to close the Sample Set Management window.

Delete a sample set

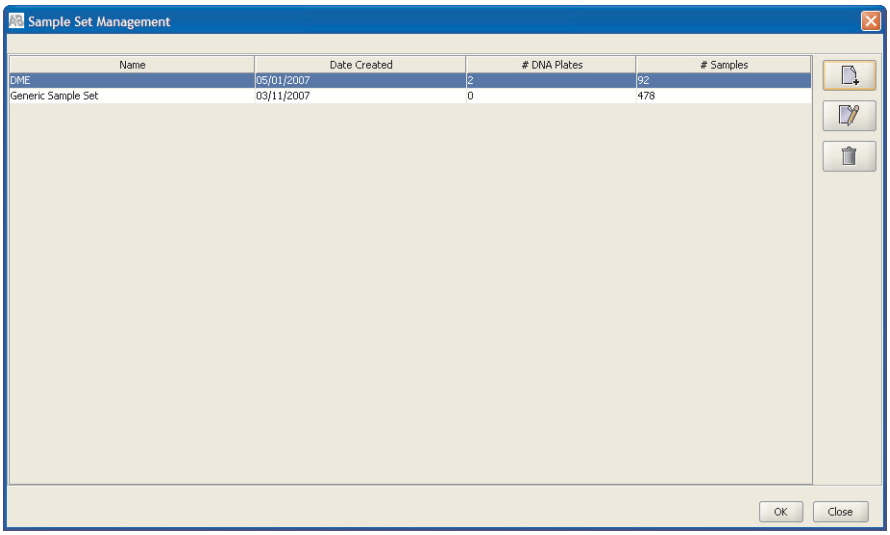
You can only delete sample sets that are not being used by any studies in the database.

1. From the main window toolbar, select **Tools** ▸ **Manage Sample Sets**.



2. In the Sample Set Management window:
   a. Select the sample set you want to delete.
   b. Click  **Delete an existing sample set**.

Notes



3. At the prompts, click **Yes** and **OK** to delete the sample set from the AutoCaller software database.

   Note: If a study is using the sample set information, the software displays an error message and does not delete the sample set.

4. Click **Close** to close the Sample Set Management window.

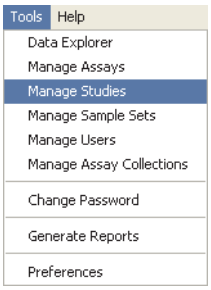
Create a study using a sample set

Once you create (page 105) and import (page 106) a sample set file into the AutoCaller software, you can create a study using the sample set.

IMPORTANT! You must create and import the sample set file into the AutoCaller software database *before* you create the study.

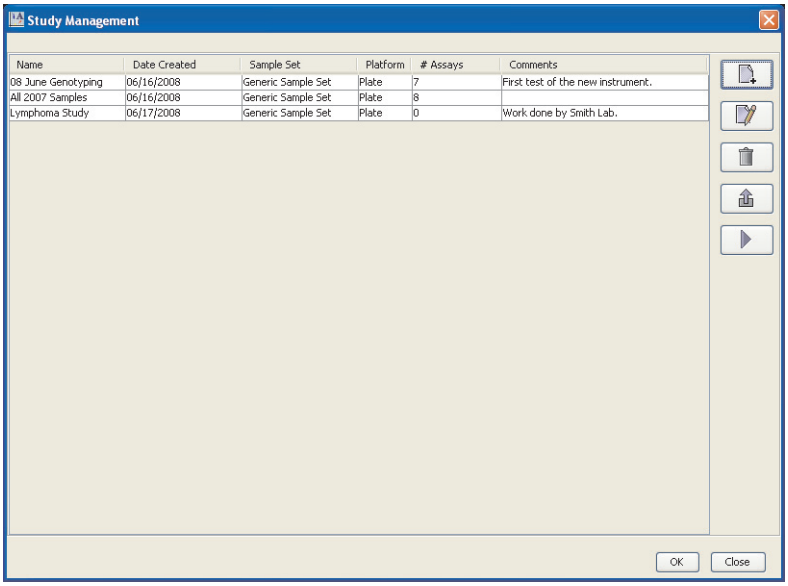
Create a study using a sample set

1. From the main window toolbar, select **Tools** ▸ **Manage Studies**.



Notes

2. In the Study Management window, click  **Add a new study to the database**.



3. In the Study Creation dialog box, enter basic study information:

   a. In the Enter Study Name field, enter a name for your study that is descriptive and easy to remember.

   b. From the Choose Platform drop-down menu, select **Plate** to indicate that you are using a 96- or 384-well reaction plate.

   Note: Choosing a platform is only for tracking purposes. The platform selection does not affect data analysis.

   c. From the Choose Sample Set drop-down menu, select the appropriate sample set.

   d. In the Choose Target Populations pane, select one or more populations.

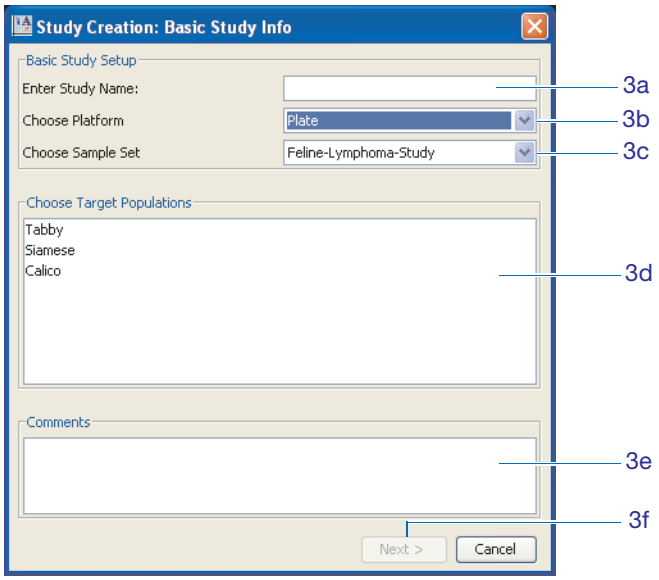
   Note: Press **Ctrl+click** or **Shift+click** to select multiple populations.

   Note: The software calculates statistics for the selected populations. The calculated statistics appear in the Statistics tab of the Analyze window. For more information, see "Statistics tab" on page 67.

   e. *(Optional)* Enter any comments in the Comments field.

   f. Click **Next**.

Notes



4. Complete the Automation Settings dialog box:

   a. To edit a value for a criterion, double-click the **Value** cell, then enter the desired value.

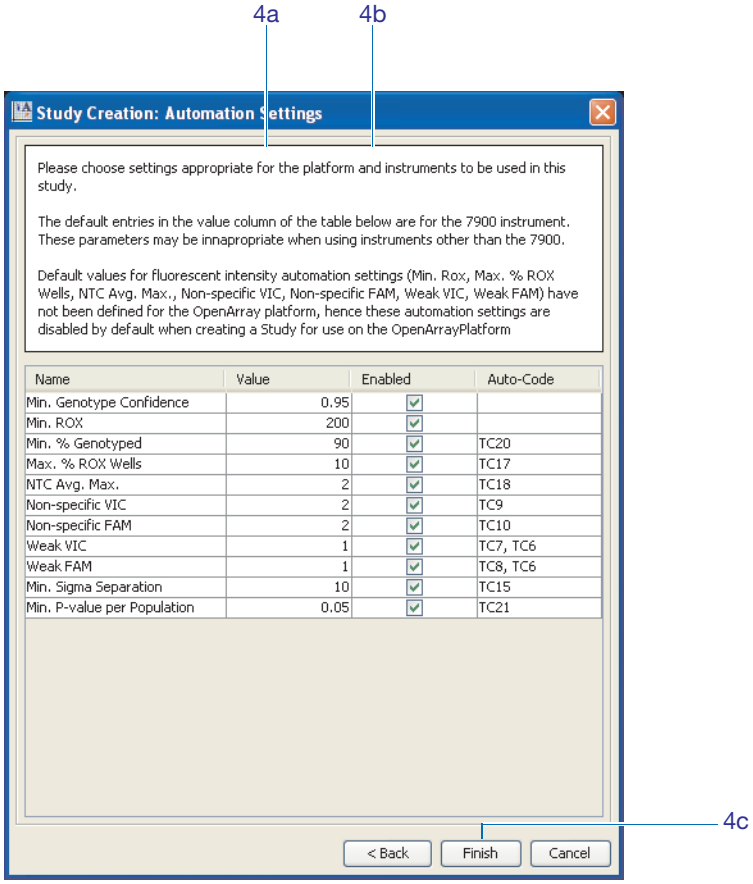
   Note: Applied Biosystems recommends accepting the default values for the analysis criteria.

   b. If you want the software to:
      - Apply a criterion to the study, select the **Enabled** check box.
      - Ignore a criterion, deselect the **Enabled** check box.

Note: The types of analysis criteria (Name column) and the Auto-Codes are predefined in the AutoCaller software and cannot be edited. For more information, see "About the automation settings" on page 22.

   c. Click **Finish**.

Notes



5. At the prompts, click **OK** to save the new study.
6. Click **Close** to close the Study Management window.

Notes

Notes

B

Manage *.xml Files

This appendix covers:

Appendix overview

This appendix explains how to manage *.xml files.

Workflow

Manage *.xml Files (Appendix B)

1. Export a study in *.xml format.
2. Import *.xml files into a study.

About *.xml files

You can use the Applied Biosystems AutoCaller™ Software to export an entire study in *.xml format. Studies that have been exported in *.xml format can then be imported back into the AutoCaller software. The *.xml files are useful when you want to share studies with other laboratories that use the AutoCaller software.

Required user levels

To perform the procedures in this chapter, log in to the AutoCaller software as a member with the required User Level. The required User Level is indicated at the beginning each section in this chapter. For information on User Levels, see "About user levels" on page 3.

Notes

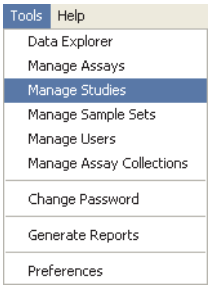
Export a study in *.xml format

Required user levels

To perform the procedures in this section, log in to the AutoCaller software at any User Level.

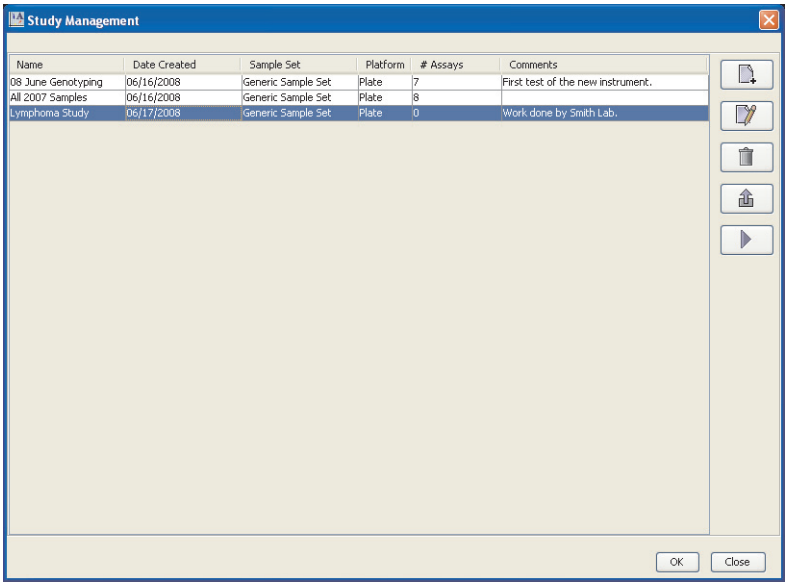
Export a study

1. From the main window toolbar, select **Tools ▸ Manage Studies**.



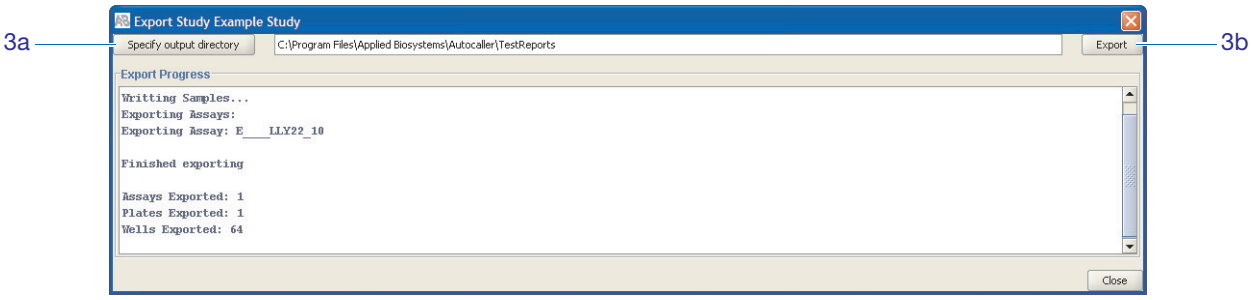
2. In the Study Management window, select the study you want to export, then click **Export the selected study**.

Note: The AutoCaller software does not export any assays in the study that have a status of NEW.



3. Complete the Export Study dialog box:
   a. Click **Specify output directory**, then browse to and select a destination folder.
   b. Click **Export**. A list of assays scrolls by as the export progresses. When the export is finished, the software displays the number of assays, plates, and wells that were exported.
   c. Click **Close** to close the Export Study window.

Notes



4. Open the destination folder to confirm that the export was successful. The AutoCaller software produces two files:
   - An *.xml file containing all data from the study.
   - A *.txt file containing a summary of the export.

   The file names include the report type (*export* and *export-log*), the database name, the study name, and the date and time the report was exported.

5. To view the export summary, open the *.txt file in a spreadsheet or simple text application.

Notes

Import *.xml files into a study

When you import *.xml files into the AutoCaller software database, the software:

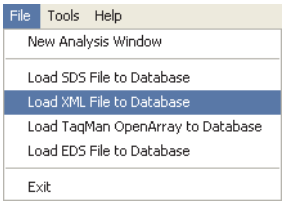
- Imports all run data associated with the assays directly into a study. You can use the software to search studies for assays, then view and edit the assay run data. For more information, see Chapter 4 on page 55.
- Imports the assay names/ID numbers into the Assay Management window. For more information, see "Edit assay information" on page 41.

Required user levels

To perform the procedures in this section, you must log in to the AutoCaller software at the Administrator or Scientist User Level.

Import an *.xml file

1. From the main window toolbar, select **File ▸ Load XML to Database** to open the Load Setup dialog box for importing *.xml files.



2. Deselect the **Conduct Auto-analysis** check box.

   Note: To ensure that all *.xml files are imported into the AutoCaller software database exactly as they were exported, the software does *not* conduct auto-analysis (that is, the software does not call the genotypes for *.xml files on import). The software does not perform auto-analysis even if you select the **Conduct Auto-analysis** check box. However, you should deselect the **Conduct Auto-analysis check box** so that all assays from the *.xml file are given the status *NEW* (for more information, see "Status menu" on page 70).

3. From the Choose Study drop-down menu, select the study you want the *.xml file imported into.

   Note: You can import the same *.xml file into more than one study.

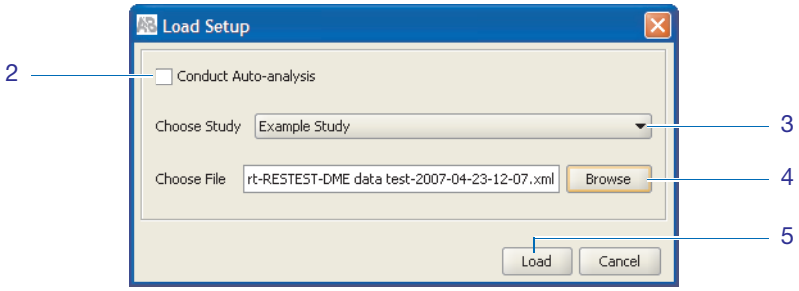
   Note: If you select **Use study indicated in the file**, the AutoCaller software displays an error message and does not import the *.xml file.

4. Click **Browse** to navigate to and select the file or folder you want to import. The file or folder name is displayed in the Choose File field.

   Note: If you select a folder, all *.xml files in the folder are imported.

Notes

5. Click **Load** to import the selected file or folder into the AutoCaller software. The Loading XML File dialog box appears while the file is being imported.



6. When the status bar in the Loading XML File dialog box displays *100%*:

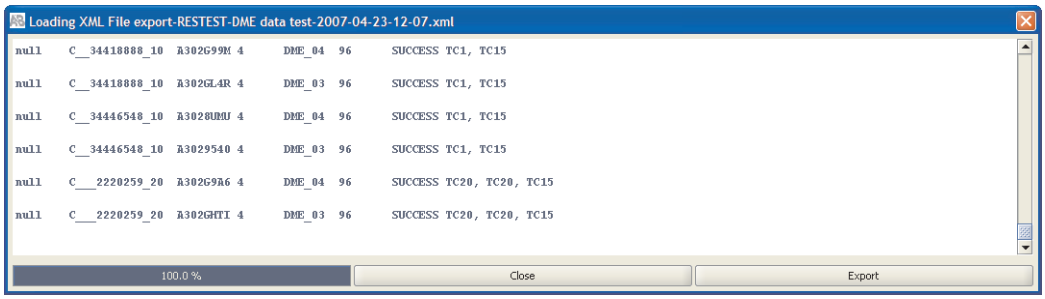
   a. Review the information for each assay:

Column	Description
Assay Name	The assay name or ID number.
Plate	The name or barcode of the reaction plate in which the assay was run. The name or barcode is entered by the user in the SDS file (page 7).
DNA Plate	The name or barcode of the source DNA plate (that is, the DNA plate that was used to seed the reaction plate): • *<DNA plate name>* – The software displays the source DNA plate name if the user included the name in the file (page 7) or in the sample set file (page 105). • *No DNA Plate* – The software displays *No DNA Plate* for any reaction plates that do not have a name or multiplate assigned.
Wells	The number of wells per assay.
Status	SUCCESS or ERROR: • If SUCCESS is displayed for all assays, the software imported the *.xml file(s) successfully. • If ERROR is displayed for one or more assays, the software does not import the *.xml file(s).
Assay Codes	The Auto-Code(s) automatically or manually assigned to an assay, as defined by the analysis criteria. For a list of Auto-Codes and the corresponding analysis criteria, see page 22.

   b. (*Optional*) Click **Export** to export and archive the information in the Loading dialog box.

   c. Click **Close**.

Notes



To search for assays and view/edit data, see Chapter 4 on page 55.

Notes

Notes

C

Software Warranty Information

This appendix covers:

Computer configuration

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems reserves the right to decline support for or impose extra charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers or processing units, installing unauthorized hardware or software may void the Warranty or Service Plan.

Limited product warranty

Limited warranty

Applied Biosystems warrants that for a period of ninety (90) days from the date the warranty period begins, its Applied Biosystems AutoCaller™ Software will perform substantially in accordance with the functions and features described in its accompanying documentation when properly installed on the instrument system for which it is designated, and that for a period of ninety (90) days from the date the warranty period begins, the tapes, diskettes, or other media bearing the software product will be free of defects in materials and workmanship under normal use. If buyer believes that it has discovered a failure of the software to satisfy the foregoing warranty, and if buyer notifies Applied Biosystems of such failure in writing during the ninety (90) day warranty period, and if Applied Biosystems is able to reliably reproduce such failure, then Applied Biosystems, at its sole option, will either (i) provide any software corrections or "bug-fixes" of the identified failure, if and when they become commercially available, to buyer free of charge, or (ii) notify buyer that Applied Biosystems will accept a return of the software from the buyer and, upon such return and removal of the software from buyer's systems, terminate the license to use the software and refund the buyer's purchase price for the software. If there is a defect in the media covered by the above warranty and the media is returned to Applied Biosystems within the ninety (90) day warranty period, Applied Biosystems will replace the defective media. Applied Biosystems does not warrant that the software will meet buyer's requirements or conform exactly to its documentation, or that operation of the software will be uninterrupted or error free.

Warranty period effective date

Any applicable warranty period under these sections begins on the earlier of the date of installation or ninety (90) days from the date of shipment for software installed by Applied Biosystems personnel. For all software installed by the buyer or anyone other than Applied Biosystems, the applicable warranty period begins the date the software is delivered to the buyer.

Warranty claims

Warranty claims must be made within the applicable warranty period.

Notes

Warranty exceptions

The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation outside of the environmental or use specifications, or not in conformance with the instructions for the instrument system, software, or accessories; improper or inadequate maintenance by the user; installation of software or interfacing, or use in combination with software or products, not supplied or authorized by Applied Biosystems; and modification or repair of the product not authorized by Applied Biosystems.

The foregoing provisions set forth Applied Biosystems' sole and exclusive representations, warranties, and obligations with respect to its products, and Applied Biosystems makes no other warranty of any kind whatsoever, expressed or implied, including without limitation, warranties of merchantability and fitness for a particular purpose, whether arising from a statute or otherwise in law or from a course of dealing or usage of trade, all of which are expressly disclaimed.

Warranty limitations

The remedies provided herein are the buyer's sole and exclusive remedies. Without limiting the generality of the foregoing, in no event shall Applied Biosystems be liable, whether in contract, tort, warranty, or under any statute (including without limitation any trade practice, unfair competition, or other statute of similar import) or on any other basis, for direct, indirect, punitive, incidental, multiple, consequential, or special damages sustained by the buyer or any other person or entity, whether or not foreseeable and whether or not Applied Biosystems is advised of the possibility of such damages, including without limitation, damages arising from or related to loss of use, loss of data, failure or interruption in the operation of any equipment or software, delay in repair or replacement, or for loss of revenue or profits, loss of good will, loss of business, or other financial loss or personal injury or property damage.

No agent, employee, or representative of Applied Biosystems has any authority to modify the terms of this Limited Warranty Statement or to bind Applied Biosystems to any affirmation, representation, or warranty concerning the product that is not contained in this Limited Warranty Statement, and any such modification, affirmation, representation, or warranty made by any agent, employee, or representative of Applied Biosystems will not be binding on Applied Biosystems unless in a writing signed by an executive officer of Applied Biosystems.

This warranty is limited to the buyer of the product from Applied Biosystems and is not transferable.

Some countries or jurisdictions limit the scope of or preclude limitations or exclusion of warranties, of liability, such as liability for gross negligence or willful misconduct, or of remedies or damages, as or to the extent set forth above. In such countries and jurisdictions, the limitation or exclusion of warranties, liability, remedies or damages set forth above shall apply to the fullest extent permitted by law, and shall not apply to the extent prohibited by law.

Notes

Notes

Glossary

Administrator — User Level in the Applied Biosystems AutoCaller™ Software. Users at the Administrator User Level can:

- Install the AutoCaller software
- Manage users
- Manage studies (includes deleting studies)
- Manage assay information and assay collections
- View and edit data
- Publish data
- Manage sample sets
- Manage *.xml files

Note: All User Levels are assigned by the users at the Administrator User Level.

call — The genotype assigned to a sample:

- 11 – Homozygous VIC® dye
- 12 – Heterozygous
- 22 – Homozygous FAM™ dye
- 00 – Undetermined genotype

Cartesian coordinates — A two-dimensional coordinate system in which each point on a plane is expressed as a distance from the two axes. In AutoCaller software, genotyping results displayed in Cartesian coordinates plots Rn for the VIC dye on the X axis and Rn for the FAM dye is on the Y axis.

confidence value — The likelihood that the point belongs to the cluster (the cluster is defined by the software).

EDS file — A file created by either the 7500 Software v2.0 (or greater) or StepOne Software. The EDS file represents a single reaction plate. Data from the EDS files can be imported into the Applied Biosystems AutoCaller Software database.

experiment — The entire process of performing a run on a real-time PCR instrument, including file setup, run, and analysis.

MAF — Minor allele frequency. The minor allele is the least frequent of the two alleles.

normalized reporter — Fluorescence signal from the reporter dye normalized to the fluorescence signal of the passive reference.

polar coordinates A two-dimensional coordinate system in which each point on a plane is expressed as an angle and a radius. In AutoCaller software, genotyping results displayed in polar coordinates plots the angle on the Y axis and $\log_{10}$(radius) on the X axis. When genotyping results are plotted in polar coordinates, the radius is a single value that correlates well with genotype.

Production User Level in the Applied Biosystems AutoCaller Software. Users at the Production User Level can:

- View data
- Publish data

Note: User Levels are assigned by the users with the Administrator User Level.

p-value Assays for which the samples have a p-value < 0.05 are 95% likely to have genotype frequencies that do not conform to Hardy-Weinberg Equilibrium (HWE) expectations. That is, there are too few homozygotes or too few heterozygotes.

Rn See normalized reporter.

Scientist User Level in the Applied Biosystems AutoCaller Software. Users at the Scientist User Level can:

- Manage studies (does not include deleting studies)
- Manage assay information
- View and edit data
- Publish data
- Manage sample sets
- Manage *.xml files

Note: All User Levels are assigned by the users at the Administrator User Level.

SDS file A file, created in the SDS software, that represents a single reaction plate. Data from an SDS file can be imported into the Applied Biosystems AutoCaller Software database. Also called a *plate document*.

sigma separation The measure of cluster separation and cluster tightness.

study A subset of data in the Applied Biosystems AutoCaller Software database that consists of a set of DNA samples run against a number of different TaqMan® genotyping assays. You can create multiple studies in the AutoCaller software database to organize your data based on projects and experiments.

validation run An experiment in which you run new assays on known DNA samples in order to establish assay performance.

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Worldwide Sales and Support

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at **www.appliedbiosystems.com**.

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